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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

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PHYSIOLOGY

VERY SLOW RHYTHMIC VARIATIONS IN THE POTENTIAL OF THE NUCLEI OF THE HYPOTHALAMUS AND THE THALAMUS

N. A. Aladzhalova and A. V. Kol'tsova

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(Received March 28, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

It has been established [1, 2] that besides the electrical activity which is usually revealed by the electrocorticogram, in various areas of the brain it is possible to register very slow periodic variations in potential, with a frequency of 5-8 per minute and an amplitude of 0.3-0.8 mv. We have called these "hyperslow" or "infraslow" [3]. Investigations [1-7] have shown that in a number of cases there is a definite connection between the phase of the very slow wave and the pattern of the electrocorticogram: for example periodic outbursts of rapid activity are observed at one of the extremes of the very slow variation [3]; in other cases intensification of the very slow rhythms accompanies synchronization of the activity of the neurones [4, 7].

The very slow variations in the intact animal are asynchronous in different areas of the hemispheres and they may have different parameters in the upper and lower layers of the cerebral cortex [5]. In a state of general anesthesia (with ether and barbiturates) they undergo depression, whereas by action on certain links in metabolism, for instance by cholinesterase inhibitors or by oxygen lack, intensification of the very slow rhythms ensues [4, 7]. Increase in the regularity and amplitude of the very slow variations is also found during prolonged stimulation of the receptors of the visual and taste analyzer [6].

Influences affecting the region of the hypothalamus as a rule stimulate the very slow variations in potential in the cerebral cortex [3, 4, 6], for instance a local injection of acetylcholine into the region of the hypothalamus leads to an increase in the regularity and amplitude (three-fold) of the very slow waves in the cerebral cortex [7]. In order to understand the mechanism of this stimulation we considered it desirable to investigate the presence of very slow variations in the structures of the hypothalamus and the thalamus.

EXPERIMENTAL METHOD

Twenty-four experiments were performed on 13 waking rabbits with impressed electrodes. To the surface of the sensomotor zone of the cerebral cortex of one or both hemispheres in each rabbit were applied plated silver electrodes for bipolar leads, 4 mm in diameter and at a distance of 6 mm between their centers. In addition two or three bipolar electrodes were impressed into the nuclei of the thalamus and hypothalamus of each rabbit. In all 20 experiments to record the potential in the nuclei of the thalamus were performed and 10 in the hypothalamus. As leads from the subcortical nuclei nichrome electrodes were used, 50-100 μ in diameter. In the case of a bipolar lead two such electrodes were fixed together with a bakelite varnish in such a way that the distance between the tips of the leads was 150 μ. In these conditions both points of the leads could be in the same nucleus of the thalamus or hypothalamus. The same electrodes were used for stimulation of the subcortical nuclei. In the case of a unipolar lead from the subcortical structures, an indifferent plated electrode was applied to the surface of the cerebral cortex in an area from which very slow variations in potential were absent. As a control of the state of the nucleus the electrothalamograms and hypothalamograms were recorded. The position of the electrode was determined by a control histological examination of the brain (thickness of section 40 μ). Electrical stimulation was carried out by rectangular impulses with a frequency of 50 cps and

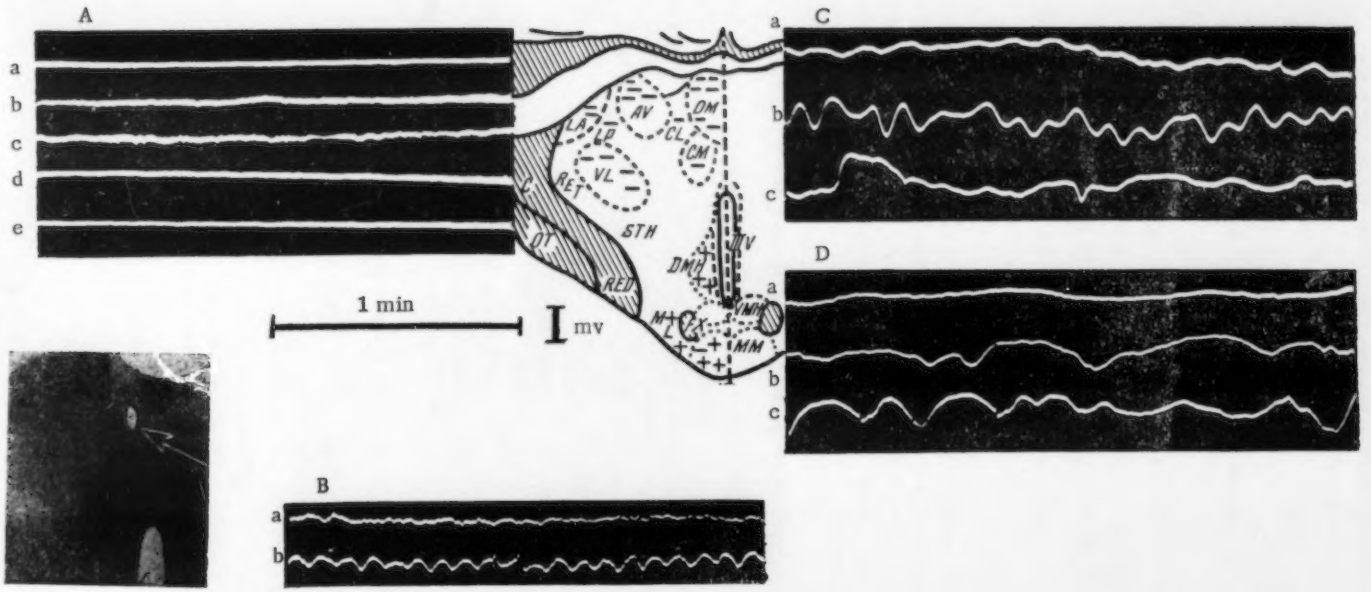

Fig. 1. Very slow variations in potential in the nuclei of the hypothalamus (+) and their absence from the nuclei of the thalamus (−). A) Lead from the thalamus: a) nucleus lateralis posterior (LP); b) nucleus medialis dorsalis (DM); c) nucleus lateralis anterior (LA); d) nucleus ventralis lateralis (VL). B) Very slow variations in potential in the DM of the thalamus before (a) and 95 minutes after (b) injection of atropine intravenously (10 mg/kg) − see section of the brain. C and D) Examples of very slow variations in the sensomotor cerebral cortex and in the premamillary region of the hypothalamus (two different rabbits): a) cortex of the right hemisphere; b) cortex of the left hemisphere; c) premamillary region of the left side of the hypothalamus (irregular very slow variations).

amplitude of 2 v, which did not cause any motor reaction. The duration of one stimulus varied from 20 seconds to 3 minutes. Electrical stimulation of the region of the hypothalamus was carried out in 12 experiments and stimulation of the lateral and medial thalamic nuclei in 10 experiments, and in 5 experiments intravenous and subcutaneous injections of strychnine were given. The potentials were recorded by means of a direct current amplifier [1] with a symmetrical input and a 5 T loop output.

EXPERIMENTAL RESULTS

In Fig. 1 are indicated in conventional form in one plane the outlines of the nuclei of the thalamus and hypothalamus in which the electrodes were impressed. The − sign indicates points in which very slow potentials were not found, while the + sign indicates where these very slow rhythms were present. In waking rabbits, subjected to no form of activity apart from the operation of insertion of the electrodes, no very slow rhythms were present in any of the 20 cases in the thalamic nuclei. In Fig. 1, A are given tracings from both the lateral and medial nuclei of the thalamus; the character of the electrograms recorded from these nuclei was proof of their preservation of function. Very slow rhythms could not be detected in the nuclei of the thalamus with either bipolar or unipolar leads. However, in the region of the premamillary hypothalamus, in the dorsal medial and ventral medial nuclei, in 9 cases out of 10 irregular very slow variations with a frequency of 5-7 oscillations per minute and an amplitude of 0.3 mv were recorded. In tracings from the sensomotor zone of the cerebral cortex, very slow rhythms were also present in 10 of 13 rabbits. In Fig. 1, C and D are shown the very slow variations in potential in the premamillary region of the hypothalamus and in symmetrical zones of the cortex of both hemispheres. In these experiments the very slow variations in the cerebral cortex were more clearly shown on the side in which the hypothalamic electrodes were impressed. This may be the result of the stimulating effect of the electrodes [3].

Ether anesthesia leads to complete depression of the very slow variations in both the hypothalamus and the cerebral cortex.

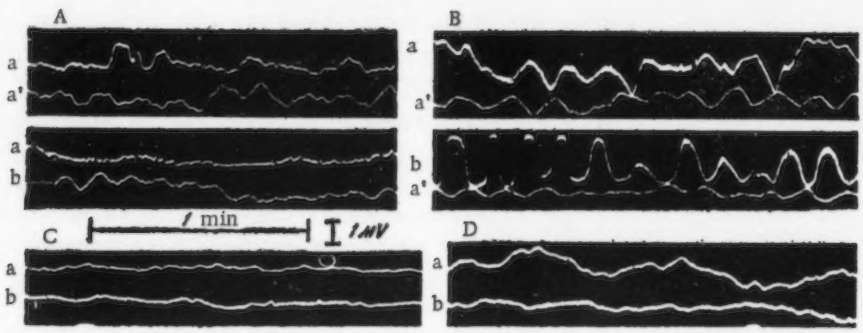

Fig. 2. The influence of electrical stimulation of the hypothalamic and thalamic nuclei on the very slow variations in the potential in the cerebral cortex and the hypothalamus. A) Two-channel tracing before stimulation of the doral medial nucleus of the hypothalamus (DMH); B) the same afrer prolonged stimulation of the DMH; a) DMH on the rightside; a') sensomotor zone of the left hemisphere; b) sensomotor zone of the right hemisphere; C and D) examples of absence of intensifying effect from the thalamus on the very slow variations in the potential of the cerebral cortex. C) Sensomotor cortex before (a) and after (b) stimulation of the thalamus − nucleus centralis pars lateralis (CL) on the ipsilateral side; D) before (a) and after (b) stimulation of the DM of the thalamus.

The irregular variations in potential in the hypothalamus may, as a result of certain influences, become more regular in amplitude and frequency. A stimulating factor of this sort is electrical stimulation. Stimulation of the dorsal medial nucleus of the hypothalamus (DMH) lasting for 2-3 minutes may cause excitation of this region, as judged by the increase in the rapid activity on the hypothalamogram (to 12 cps), although in

some cases there were no visible changes in the rapid activity. At the same time only 2 minutes after stimulation it was possible to observe clear intensification of the very slow variations at first in the hypothalamus itself and later (after 20 minutes) in the ipsilateral cerebral cortex, and still later (after 120 minutes) in the contralateral cortex.

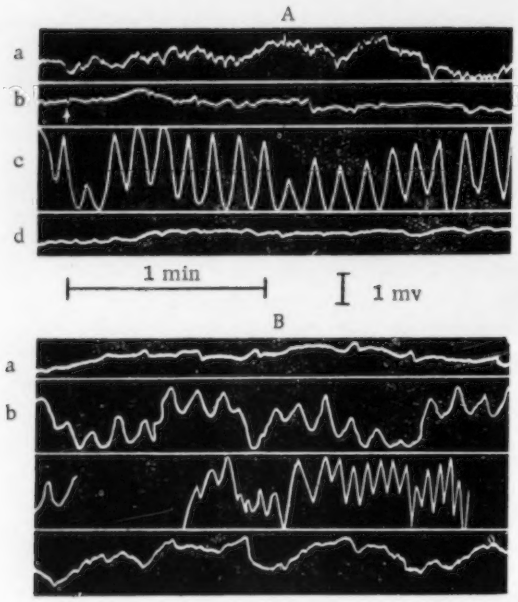

Fig. 3. The effect of strychnine on the very slow potential in different structures. A) Premammilary region of the hypothalamus; a) before injection; b) 17 minutes after injection of strychnine in a dose of 0.5 mg/kg intravenously (— convulsion); c) 25 minutes afterwards (regular rhythm of 8 cycles per minute); d) after 80 minutes (depression of the rhythm). B)The cerebral cortex in the same experiment; a) before injection of strychnine; b) 40 minutes after injection (rhythm of 8 cycles per minute); c) after 47 minutes (rhythm of 16 cycles per minute); d) after 60 minutes.

However, in order to obtain clearly the effect of intensification of the very slow variations in the hypothalamus and cerebral cortex, it was sometimes necessary to use stimuli to the hypothalamus which were repeated several times, as a result of which synchronous very slow rhythms arose in symmetrical areas of the hemispheres and in the hypothalamus. In Fig. 2 A and B are given two-channel records from the dorsal medial nucleus of the hypothalamus and the cerebral cortex of both hemispheres before stimulation (A) and after stimulation three times of the DMH (B) lasting for up to 30 seconds. As a result, synchronous oscillations appeared in all three regions in a frequency of 5 per minute and an amplitude of 1 and 1.5 mv.

Electrical stimulation of the thalamic nuclei: central lateral (CL), dorsal medial (DM), lateral anterior (LA) and lateral ventral (LV) did not produce this effect and could even cause depression of the very slow oscillations in the cerebral cortex (Fig. 2 C and D).

Injection of strychnine increased the frequency and amplitude of the very slow variations in the hypothalamus and the sensomotor zone of the cerebral cortex.

In Fig. 3, A it is seen that after intravenous injection of strychnine (0.5 mg/kg) a phase of depression of the very slow variations appears after 15 minutes in the premamillary region of the hypothalamus, associated with which are observed convulsions, temporary respiratory paralysis and, in consequence of this, asphyxia. After recovery from this state, 25 minutes after the injection, a very regular slow rhythm arises in the region of the hypothalamus, with a frequency of 8-9 cycles per minute and an amplitude rising to 2.5 mv. Forty minutes later this same rhythm was recorded in the cerebral cortex (Fig. 3, B), and after 7 minutes the frequency of the rhythm increased to 16 cycles per minute. After an hour the very slow variations in both the hypothalamus and the cerebral cortex became weaker.

In another experiment in which strychnine was injected subcutaneously in a dose of 0.6 mg/kg, without complication by asphyxia after brief convulsions associated with depression of the very slow variations, after 40 minutes there appeared in the hypothalamus a rhythm with a frequency of 6 per minute and an amplitude of 2 mv; after 75 minutes there were recorded in the cerebral cortex variations at the rate of 9 per minute with an amplitude of 1 mv; there was no effect in the anterior lateral nucleus of the thalamus (LA).

The absence of very slow variations in the potential of the thalamus in the intact animal and their presence in the hypothalamus, and also the possibility of their appearance in the thalamus after action on the metabolism of the brain, evidently indicate the existence of an intimate connection between the phenomenon of very slow variations and peculiarities of the metabolic processes.

A specific property of the hypothalamus is its regulating action on the very slow potential of the cerebral

cortex. This is clearly observed during electrical stimulation of the dorsal medial and ventral medial nuclei of the hypothalamus.

During the action of strychnine the change in the parameters of the very slow rhythms in the hypothalamic region outstrips the changes in the cerebral cortex by 10-30 minutes. This time sequence admits the possibility of a nervous mechanism of regulation of the very slow rhythms in the cerebral cortex by the hypothalamus, since it is at roughly this same interval of time after injection of strychnine into the nucleus of the hypothalamus that discharges appear in the cerebral cortex [8]. It may be supposed that the mechanism of regulation consits of changes in the local chemical gradients in the structures of the cerebral cortex.

The appearance of synchronous pulsation of the very slow variations in different areas of the hemispheres and in the hypothalamus itself after stimulation indicates the onset of a generalized effect, during which the separate regions of the cerebral cortex may take part in synchronized activity.

Equality of the functional potentials in the different zones is organized in this case through a neuroendocrine mechanism.

Synchronization of the very slow variations evidently reflects the tuning of different areas of the hemispheres to a single metabolic level. At the same time the electrical field created in the cortex by the intensified very slow variations of potential extended to cover a considerable mass of dendrites, and by affecting the electrotonic potentials of the dendrites, it was able to alter periodically the excitation of the system of cortical neurones [4, 9]. Evidently the very slow potential is one of the links in the mechanism linking the neuroendocrine function of the brain and its electrical activity, thereby taking part in the integrative action of the brain.

SUMMARY

Infraslow oscillations (8 cycles per minute, 0.5 – 1 mv) formerly revealed in the rabbit's brain cortex, were also registered in the premammillary hypothalamus. This infraslow rhythm was absent in the thalamic nuclei of intact animal, but could be revealed after acting upon the brain metabolism by strichnine or atropine.

Electric stimulation of hypothalamic dorsal-medial and ventral-medial nuclei increases the amplitude and the regularity of the infraslow rhythm in the sensomotor brain cortex and the hypothalamus proper.

After strychnine injection the intensification of the infraslow fluctuations appears in the hypothalamus 10-30 minutes earlier than in the brain cortex.

Prolonged stimulation of hypothalamus may cause generalized infraslow oscillations synchronic in different portions of the brain. Creating a definite gradient of electric field in the brain cortex the infraslow potential affects the excitability of neurons, reflecting the association between the neuroendocrine function and the electric activity of the brain. Infraslow oscillations are one of the links in the integrative brain activity.

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*In Russian.

AN ELECTROPHYSIOLOGICAL INVESTIGATION INTO THE AFFERENT CONNECTIONS OF THE THYROID GLAND WITH THE CENTRAL NERVOUS SYSTEM

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The question of the afferent connections of the thyroid gland with the central nervous system has so far received little attention. In morphological papers are described various forms of receptor endings both in the parenchyma of the gland and in the walls of the blood vessels [1, 4, 5, 7, 8, 9 and others]. However their functional importance in the activity of the thyroid gland has not yet been explained.

The purpose of the present investigation was to establish by an electrophysiological method the presence of afferent impulsation from the receptors of the thyroid gland during the action of various agents on this organ.

Electrophysiological data in respect of afferent signals from the receptors of the thyroid gland are not available, with the exception of a brief reference in the paper by Andrew [6]. This author observed an outburst of impulses in the superior laryngeal nerve during mechanical stimulation of the thyroid gland. On the basis of this observation he put forward the view that mechanoreceptors were present in the thyroid gland.

EXPERIMENTAL METHOD

Experiments were carried out on 90 adult cats weighing from 2.5 to 5 kg. An intramuscular injection of a solution of sodium amytal in a dose of 70-80 mg/kg body weight was given to each animal. A longitudinal skin incision was made in the neck. The thyroid gland was exposed by retraction of the sternothyroid, sternohyoid and cricothyroid muscles to the side. The topography of the thyroid nerve was very variable and in some cases it could not be found. For taking a lead of the afferent impulses the nerve branch which was isolated along the course of the thyroid artery was usually utilized. This branch was divided at the point where the thyroid artery emerged from the carotid. The peripheral end of the branch was seized in a ligature and laid on silver electrodes, the distance between the electrodes being 3-4 mm. The potentials of the afferent impulses were passed through an amplifier to a cathode ray oscillograph. The frequency characteristic curve of the amplifier was rectilinear between 10 and 1500 cps.

EXPERIMENTAL RESULTS

In the majority of the experiments with no form of action on the thyroid gland afferent impulses were not present in the thyroid nerves (Fig. 1, a, e). In a series of experiments the impulses took the form of irregular rapid oscillations with an amplitude of 5 to 50 μv (Fig. 1, c, g). During local warming of the thyroid gland by application of cotton wool soaked in warm (about 40°C) physiological saline, in all the experiments afferent impulses were found to appear, or those already in existence were strengthened (Fig. 1, b, d, f). The stream of impulses lasted until the cotton wool became cool. In some of the experiments grouping of impulses to the rhythm of the pulse beats was sometimes observed in response to warming the gland, which may indicate some connection between these impulses and the vascular receptors. Each group consisted of 2-8 separate

oscillations (Fig. 1, h). During warming of the thyroid gland, in addition to relatively rapid variations in the potential, impulses were observed which were characterized by slower oscillations in the form of biphasic waves with an amplitude of 10 to 20 μ v, in single phases (Fig. 1, f). Potentials of this type were found earlier in impulses from receptors lying in the walls of the intestine [3] and the stomach [2].

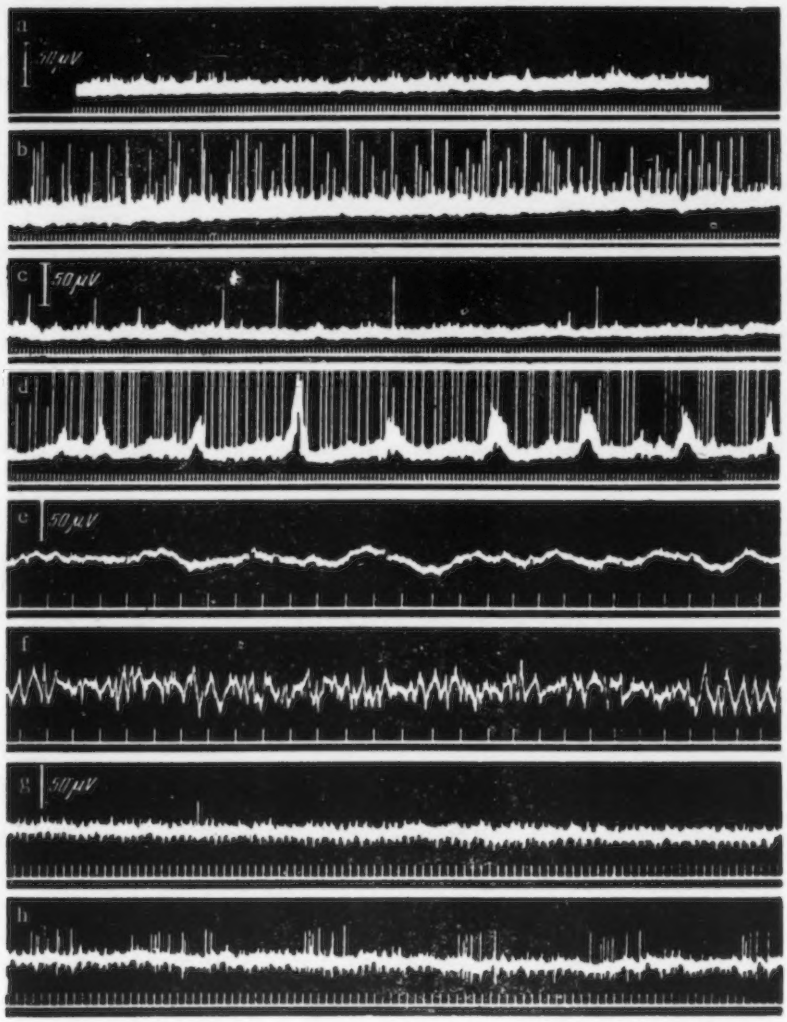

Fig. 1. Electrical activity in the peripheral end of the thyroid nerve before and during warming of the thyroid gland. a, c, e, g) Before warming; b, d, f, h) during warming of the gland. In this and all subsequent illustrations the time marker records 0.02 seconds.

In the next series of experiments the action of a series of drugs which alter the state of the blood supply to the thyroid gland was studied – a 10% solution of caffeine, histamine in a dilution of $1 \cdot 10^{-5}$ and adrenalin in a dilution of $1 \cdot 10^{-4}$. These solutions were injected into the thyroid artery in the course of the blood flow in a volume of 0.5 - 1 ml. In order to prolong the action of the agent on the receptors of the gland during injection of the solution, the blood flow in the thyroid gland was artifically slowed by means of partial ligation of the carotid artery above the origin from it of the thyroid artery.

During injection of a 10% solution of caffeine into the thyroid artery, afferent impulses arose every time even though they had previously been absent. The maximum intensity of this impulsation was reached on the 2nd-3rd minute after injection of caffeine, when reddening of the gland was observed in consequence of vasodilatation. In Fig. 2, a before injection of caffeine complete absence of afferent impulses was observed. Forty-five seconds after injection of 0.5 ml of a 10% solution of caffeine isolated potentials were observed with an amplitude of 100 μ v. Two minutes after the injection of caffeine the frequency of the potentials was considerably increased (Fig. 2,b). This effect of increase in afferent impulsation in the nerves of the gland was observed for a period of 8-10 minutes after injection of caffeine.

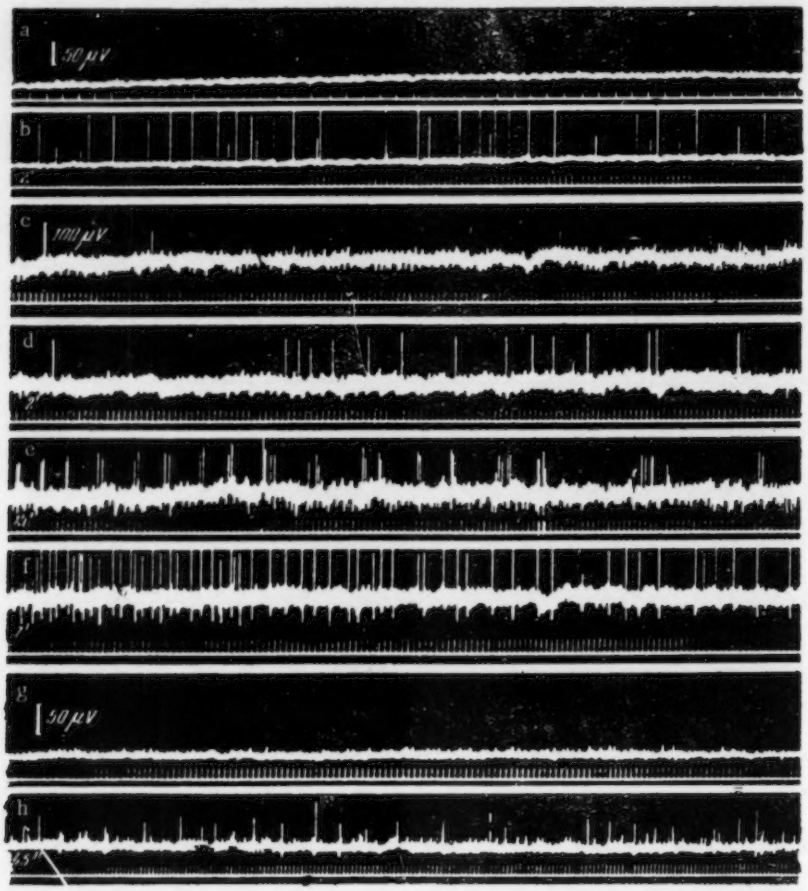

Fig. 2. Electrical activity in the peripheral end of the thyroid nerve before and after injection of caffeine, histamine and adrenalin into the thyroid artery. a) Before injection of caffeine; b) 2 minutes after injection of caffeine; c) before injection of histamine; d) 2 minutes after injection of histamine; e) 15 minutes after injection of histamine; f) 7 minutes after a second injection of histamine; g) before injection of adrenalin; h) 45 seconds after injection of adrenalin.

During injection of histamine also the amplitude and frequency of the potentials were increased.

Whereas before injection of histamine (Fig. 2, c) single oscillations with an amplitude of 25-30 μ v were rarely observed, after injection into the blood stream of 0.06 ml of histamine in a dilution of $1 \cdot 10^{-5}$ both the frequency and the amplitude of the potentials rose (Fig. 2, d). They were sometimes found to be grouped together in the rhythm of the contractions of the heart. The effect of intensification of the impulses was more

prolonged than with injection of caffeine, and it could be registered even 15 minutes after the injection of histamine (Fig. 2, e). A futher injection of histamine into the thyroid artery was again accompanied by increase in the frequency of the impulses (Fig. 2, f).

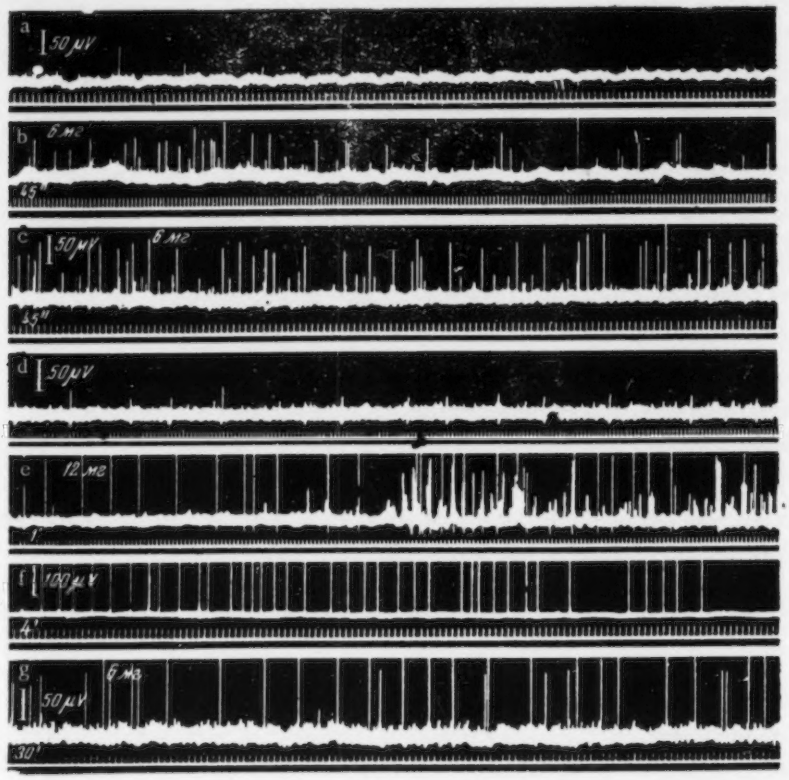

Fig. 3. Electrical activity in the peripheral end of the thyroid nerve before and after injection of thyrotropic hormone into the thyroid artery. a) Impulses before injection of the hormone; b) 45 seconds after the injection of 6 mg of thyrotropic hormone; c) 45 seconds after a second injection of the hormone in a dose of 6 mg; d) before injection of hormone; e) 1 minute after injection of 12 mg of the hormone; f) 4 minutes after the same injection with a reduction of the amplification by one half; g) impulses 30 minutes after injection of the hormone.

The action of adrenalin was considerably weaker than those of caffeine and histamine. Forty-five seconds after injection of 0.5 ml of adrenalin in a concentration of 10^{-4} into the thyroid artery a brief appearance of afferent impulses, previously absent, was observed (Fig. 2, h, g). After the injection of adrenalin a less frequent and less regular rhythm of impulses was noted in the thyroid nerve than under the influence of caffeine and histamine. From $1\frac{1}{2}-2$ minutes after the injection of adrenalin the impulses completely ceased.

In the next series of experiments the influence of a solution of thyrotropic hormone in 0.7% saline was studied. In all the experiments in which thyrotropic hormone was injected, the appearance of afferent impulses or the intensification of previously existing impulses before the action of the hormone was observed in the thyroid nerve. After injection of 6 mg of hormone an increase in the frequency of the potentials always took place and was accompanied by an increase in their amplitude (Fig. 3, a, b). This effect was observed for a period of 3-4 minutes. A futher injection of 6 mg of the hormone caused a more appreciable increase in the frequency of the impulses (Fig. 3, c) and the appearance of new oscillations, whose amplitude reached 200-220 μ v. The appearance of oscillations of this form was observed during repeated injection of thyrotropic

hormone or by increasing its dose to 12 mg. Thus in Fig. 3, d may be seen predominance of potentials with an amplitude of 25-30 μ v. One minute after injection of 12 mg of thyrotropic hormone into the thyroid artery oscillations of such a high amplitude were seen that their apices were cut off (Fig. 3, e). When the degree of amplification was reduced by half, it could be seen that their amplitude reached 200-220 μ v (Fig. 3, f).

If the time of action of the hormone on the receptors of the gland is prolonged by delaying its passage into the general circulation by means of partial clamping of the veins of the thyroid gland, the effect of intensification (increase of frequency and of amplitude) of the afferent impulsation can be observed to last for 30 minutes after the injection of 6 mg of thyrotropic hormone (Fig. 3, g). Without clamping of the veins the impulses after injection of 6 mg of the hormone returned to their original level after 4-5 minutes.

As may be seen from the results described, afferent impulses from the receptors of the thyroid gland were frequently absent before the action of certain agents. Such impulses always appeared, however, when the gland was warmed. This feature may suggest that during an acute experiment the thyroid gland was subjected to cooling, as a result of which the metabolic rate was diminished, the vessels were constricted and the functional state of the receptors of this particular organ was aggravated. During warming of the gland, not only was there local dilatation of the vessels and increase in the blood flow, but the metabolic processes in the tissues were speeded up. In consequence of this there was an increase in the excitation of the receptors of the organ, leading to a greater synchrony of their action (see Fig. 1). Increased synchronization of the work of the receptors during warming was also brought about by intensification of the variations in pulse pressure, which act as a natural stimulus for the vascular receptors.

The fact that the afferent impulses from the thyroid gland mainly reflect the state of its blood vessels is demonstrated by our experiments in which injections of caffeine, histamine and adrenalin were given. In the experiments where thyrotropic hormone was injected we observed in addition to an increase in the frequency of the potentials of the relatively rapid afferent impulses, the appearance also of specific potentials characterized by extremely high values of their amplitude. It is probable that in this case special receptors situated in the tissues of the gland, described by histologists in the form of bulb-shaped formations [8], are subjected to stimulation. However it is not impossible that there is an increase in amplitude on account of the synchronization of action of the receptors which are connected with the activity of the vascular apparatus of the thyroid gland.

SUMMARY

Experiments were performed on cats under amytal anesthesia. The potentials of afferent impulses due to various actions upon the thyroid gland, were registered in the nerve supplying the thyroid gland. Three types of afferent impulse potentials were revealed: relatively slow low voltage oscillations (in heating the gland), quick oscillations with the amplitude from 10 to 100 μ v (in heating the gland) and in injection of substances into the thyroid artery (caffeine, histamine, adrenalin, and thyrotropic hormone), and quick oscillations with the amplitude of 200-220 μ v in introduction of thyrotropic hormone into the thyroid artery.

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THE CONDITIONED REFLEX REGULATION OF THE BLOOD PRESSURE

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Changes in the blood pressure during muscular activity are well known. As long ago as in 1883, I. P. Pavlov [7] observed by recording the blood pressure of dogs during experiments of long duration that every movement of the animal is accompanied by a brief variation in the level of the blood pressure.

In the last ten years it has been shown by Soviet physiologists [1, 2, 4, 5, 6, 9, 10] that a conspicuous place in the production of these changes belongs to a reflex component, particularly during the action of the muscles of both adequate (physical work, stretching of the muscles by a load) and inadequate (electric shock, vibration, heat, chemical agents) stimuli.

N. I. Kozin [3] observed in children a conditioned reflex rise in the blood pressure to a bell and to the word "bell," reinforced by physical exercise. In modern Soviet textbooks of physiology and in some reports of investigations [8, 11] changes in the arterial pressure during imaginary work and also in the periods before starting work or activity are treated as conditioned reflex in origin. However the properties of these conditioned reflexes have received inadequate study.

In this connection we made an attempt to investigate some of the properties of the conditioned reflexes formed on the basis of unconditioned changes in the arterial pressure during stimulation of proprioceptors.

EXPERIMENTAL METHOD

The experiments were carried out on 3 dogs — Trezor, Jack and Sharik. The arterial pressure was recorded in the common carotid artery exteriorized in a skin flap by means of L. N. Deriabin's apparatus. This apparatus is so constructed that a fall in the curve of the kymogram corresponds to an increase in the arterial pressure and vice versa, and only the maximum arterial pressure can be measured.

The stimulus for the proprioceptors was a static load equal in the size to 80% of the body weight of the animals. A bag containing sand was placed on the animal's back. The conditioned stimuli were a positive electro-metronome with a frequency of 150 strokes per minute (EM_{150}) and a differential electrometronome with a frequency of 75 strokes per minute (EM_{75}). Delayed conditioned reflexes were established. The delay amounted to 10-30 seconds. The unconditioned stimulus was applied to two of the dogs for 4 minutes and to one for 2 minutes, depending on the time over which the animals were able to support the load without movement. The reaction to the positive conditioned stimulus was recorded during periodical tests of its isolated application.

At the beginning of the investigation the orientational reflexes to the apparatus and to the conditioned stimuli were extinguished, and the character of the changes in the arterial pressure under the influence of the static load revealed. In addition, by repeated measurements of the arterial pressure we were satisfied that compression of the common carotid artery by the cuff did not in itself cause any variation in its level. In this respect we confirmed the findings of Wilhelmy and his co-workers [12] that the significant variations in the arterial pressure observed at the start of its systematic measurement, after the dog has become acclimatized to

the apparatus, become smoothed out and the arterial pressure becomes constant for a given animal, although it differs from one dog to another.

EXPERIMENTAL RESULTS

The results shown in the table demonstrate that in different dogs the conditioned reflexes which we studied appear roughly the same. The formation, restoration and reorganization of the conditioned reflexes take place rapidly; they are extinguished much more slowly.

Data on the Properties of the Conditioned Reflexes Formed in Dogs on the Basis of Unconditioned Reflex Changes in the Arterial Pressure Arising Under the Influence of a Static Load

Properties of the conditioned reflexes	Number of combinations (applications) of the stimuli to the dogs		
	Trezor	Jack	Sharik
Extinction of the reaction to compression of the carotid artery with the cuff	7	9	8
Extinction of the orientational reaction to the conditioned stimuli (EM_{150} and EM_{75})	4	5	3
Formation of a conditioned reflex to EM_{150}	3–7	4–10	2–8
Extinction of the conditioned reflex to EM_{150}	14–18	16–24	9–18
Restoration of the conditioned reflex to EM_{150}	1	1	2–6
Formation of differentiation to EM_{75}	2–4	7–11	4–5
Simultaneous transformation of the signal meaning of a pair of conditioned stimuli	5–7	4–7	5–8
Reverse transformation of the signal meaning of the pair of conditioned stimuli	4–5	3–5	4–6

At the start of formation of a conditioned reflex, a positive conditioned stimulus, applied in isolation, causes considerable variations in the level of the arterial pressure, particularly at the end and immediately after cessation of the action of the stimulus. Subsequently the changes in the level of the arterial pressure arising under the influence of the stimulus approximate those which are observed during the use of the static load (Fig. 1, a). At this period the conditioned stimulus – quite independently of whether it accompanies merely the start of the action of the unconditioned stimulus or the whole period of its action – when applied in isolation fully reproduces the action of the unconditioned stimulus. Later on, repeated combinations of the conditioned and unconditioned stimuli lead to a gradual diminution in the variations in the level of the arterial pressure. Next these stimuli may completely fail to evoke any changes in the arterial pressure. Under these circumstances a diminution in the variations and disappearance of changes in the arterial pressure are observed much sooner by use of the conditioned stimulus than by actual physical loading.

During extinction of the conditioned reflex, with a maximum reaction to EM_{150} at the start of nonreinforcement, considerable fluctuations in the level of the arterial pressure are also observed. Later on these fluctuations become diminished in association with a fall in the maximum arterial pressure, and the action of the conditioned stimulus leads to a fall in the level of the arterial pressure. If the conditioned stimulus is applied without reinforcement later on, then eventually it will cause no changes in the level of the arterial pressure exceeding the limits of the ordinary variations (see Fig. 1, b).

After extinction of the conditioned reflexes they were restored, and at the maximum of the changes in blood pressure differentiation to EM_{150} was developed. On its first application, the differential stimulus (EM_{75}) produces almost the same magnitude and direction of change in the arterial pressure as does EM_{150}. In subsequent tests of the influence of the nonreinforced EM_{75} the level of the arterial pressure falls and becomes lower than its variation observed when the stimuli are not in operation. At this period an obvious reaction is observed also to the unconditioned stimulus and to positive and inhibitory conditioned stimuli (Fig. 2).

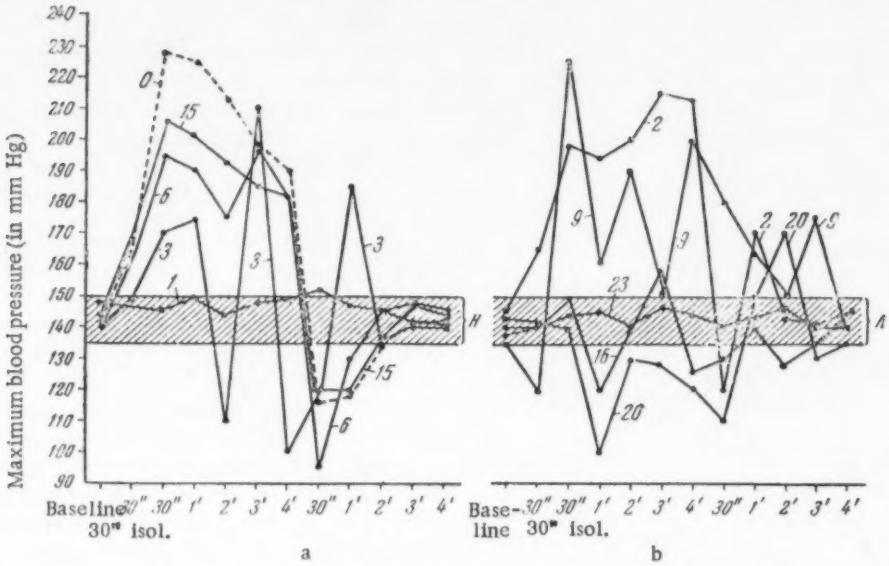

Fig. 1. Changes in the level of the arterial pressure in the dog Sharik. a) In the process of formation of the conditioned reflex; O) during the 15th combination of stimuli and during the action of EM_{150} in isolation after the 1st, 3rd, 6th and 15th combinations; b) in the process of extinction of the conditioned reflex during the 2nd, 9th, 16th, 20th and 23rd nonreinforcement; baseline – level of the initial blood pressure before this particular trial of the action of the stimulus; 30" isol. – action of the conditioned stimulus in isolation for 30 seconds; 30", 1', 2', 3', 4' – time of action of stimuli, and the later – 30", 1', 2', 3', 4' – time after their action; H) variations in the level of the blood pressure at times other than when the stimuli were applied.

Simultaneous transformation of the signal meaning of a pair of conditioned stimuli takes place rapidly. With the first combinations of the differential stimulus none of the changes in the level of the arterial pressure take place with the static load that are observed when the load is acting in isolation. The reaction to the unconditioned stimulus is inhibited by the existing differential stimulus. After 2-3 applications of these stimuli the level of the arterial pressure is increased considerably more under their combined influence than by the action of physical load alone. After 7-8 applications the changes in the arterial pressure become the same as those which took place earlier under the influence of the combination of stimuli for the consolidated conditioned reflex. Reverse transformation of the conditioned reflexes takes place rather more rapidly than the primary transformation.

We carried out "conflict" experiments on the dog Sharik. As a preliminary we applied EM_{150} and EM_{75} for 4 minutes. The reaction to these stimuli was the same as that shown in Fig. 2. For a period of 2 days we made 5 applications for 2 minutes of the positive conditioned stimulus, and immediately after these, also for 2 minutes, the inhibitory conditioned stimulus. During the first three of these actions (on the 1st day) the conditioned stimuli, during their time of action, caused changes in the arterial pressure in accordance with their signal meaning. During the subsequent two "conflicts" (2nd day) the reaction to the positive conditioned stimulus diminished but the differential stimulus caused an increase in the level of the arterial pressure. On

the 3rd and 4th day after the "conflict" the level of the arterial pressure at times when no stimuli were being applied was from 10-20 mm of mercury above normal. Usually in our experiments the level of the blood pressure fell to its initial value from 3-4 minutes after the action of the stimuli. Starting with the first conflict between the excitatory and inhibitory processes in the cerebral cortex, the level of the arterial pressure increased by a high degree and returned to its original value only after 13-15 minutes. Under the influence of a combination of the conditioned stimulus with the unconditioned, the blood pressure also rose much more than usually, and the initial level was restored after 9-11 minutes. Further observations on the changes in the level of the blood pressure under the influence of conditioned stimuli revealed, usually in the form of a neurosis, inhibitory, ultraparadoxical and equalizing phases. The reactions of the animal both to signal stimuli connected with kinesthesia and to unconditioned proprioceptive stimuli became inadequate. The animal reacted to the conditioned stimuli in accordance with their signal meaning only 10 days after the "conflict."

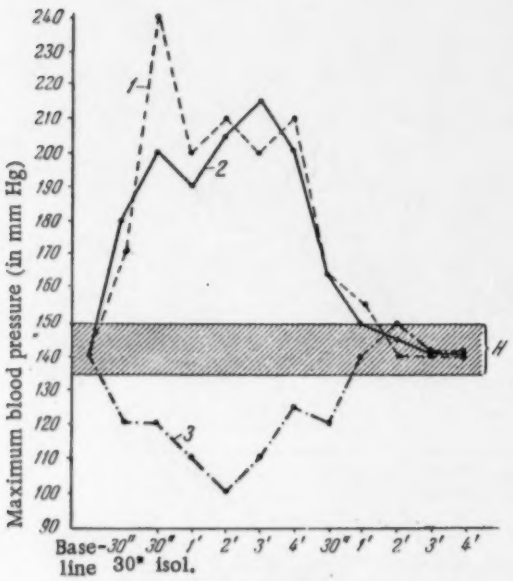

Fig. 2. Changes in the level of the blood pressure after reinforcement of conditioned reflexes in the dog Sharik. 1) during reinforcement of the conditioned stimulus by a static load; 2) during action of the positive conditioned stimulus in isolation; 3) under the influence of the differential stimulus. Symbols as in Fig. 1.

The results of the experiments which were carried out confirm the correctness of the assertion of M. R. Mogendovich, based on insufficient factual material, that "vegetative functions are subordinated to the locomotor functions during the predominant role of the higher nervous activity" ([6], p. 18). Nevertheless it is difficult to agree with this author that in the conditioned reflex reaction of the cardiovascular system to muscular work "the main part is played by the unconditioned reflex mechanism of regulation" [[6], p. 259]. M. R. Mogendovich bases this conclusion on the fact that in man a verbal signal evokes a smaller acceleration of the heart rate than does muscular exercise. In our own experiments, at the beginning of consolidation of the conditioned reflexes the conditioned stimulus caused almost the same changes in the level of the arterial blood pressure as the unconditioned. Only later on, after repeated applications of combinations of the conditioned stimulus with the unconditioned, did the reaction to the first of these develop significantly more rapidly than to the second, diminish and even disappear. In our opinion this is not evidence in favor of the predominance of the unconditioned reflex regulation of the cardiovascular system over the conditioned reflex regulation.

The results of our experiments demonstrate that the conditioned reflexes formed on a basis of unconditioned reflex changes formed on a basis of unconditioned reflex changes in the arterial pressure during stimulation of proprioceptors are of great importance in the life of the animal; it may be suggested that they are mainly responsible for bringing about rapid adaptation of the function of the cardiovascular system to the constantly changing conditions of the external environment of the animal.

SUMMARY

The conditioned reflexes formed on the basis of unconditioned changes of the blood pressure in stimulation of the proprioceptors with the aid of static load were studied in experiments on 3 dogs. It was demonstrated that the reflexes referred to above are rapidly acquired, reestablished and changed, but in comparison with this their extinction is slower. It is thus assumed that these conditioned reflexes provide the adjustment of the function of the cardiovascular system to the constantly changing conditions of the body existence.

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VASOMOTOR CONTROL MECHANISMS

COMMUNICATION I. CORRELATION OF SYSTEMIC AND REGIONAL VASOMOTOR REFLEXES DURING STIMULATION OF CERTAIN INTEROCEPTIVE ZONES

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The changes in the arterial pressure which arise during stimulation of numerous interoceptive zones are due, as we know, to a reflex alteration in the lumen of the blood vessels under the control of vasomotor fibers. These reflexes may be called systemic, since they affect the circulatory system as a whole. The systemic reflexes are an integral reaction caused by aggregation of regional vasomotor reflexes taking place in several different vascular regions.

A number of results obtained in previous investigations [4-6] led us to the view that behind the outwardly standard character of the systemic reflexes there are hidden various complexes of regional vasomotor reflexes whose specific features are determined by the appearance locally of afferent signals. During prolonged stimulation of an interoceptive zone the afferent impulses gradually lose their effectiveness in consequence of the appearance of inhibition in the central link in the reflex arc; this inhibition is, however, localized to this particular arc and does not prevent the appearance of systemic reflexes from other zones. This phenomenon permits speculation on the presence of definite "projections" of the interoceptive zones in the vasomotor regulatory centers and suggests that there are differences in the efferent structure of the systemic reflexes arising from different zones.

This is demonstrated by the results of a number of investigations. According to Bernthal and Swind [9], stimulation of the carotid and aortic chemoreceptors diminishes the blood flow in the brachial artery of dogs significantly more than in the vessels of the small intestine. T. S. Lagutina showed that stimulation of the mechanoreceptors of the urinary bladder causes a change in the pattern of impulses in the efferent fibers of the mesenteric and renal nerves and of the subcutaneous, hypogastric and cervical sympathetic nerves, and moreover the intensity and direction of the changes in the efferent impulses are regularly determined by the strength of stimulation of the mechanoreceptors. A. M. Blinova, G. N. Aronova and K. E. Serebrianik [1] discovered a somewhat differnt change in the resistance in the vascular channels of the mesenteric, femoral and renal arteries during what outwardly appeared to be a homogeneous systemic reaction – an increase in the general arterial pressure in response to stimulation of the hypothalamus or compression of the carotid arteries. A systematic study of the reflex connections between the interoceptive zones and the vessels in different organs would, in our view, aid in our understanding of the mechanisms of vasomotor control.

EXPERIMENTAL METHOD

A quantitative estimation of regional vasomotor activity is usually made with the aid of measurement of the blood flow in the vascular region under investigation. However in a simultaneous investigation of the changes in the arterial pressure (systemic reflex) and in the lumen of the vessels in individual organs (regional reflexes),

registration of the rate of the blood flow leads to indeterminate results, demanding further interpretation of the values of hemodynamic and vasomotor factors in the blood supply of the organ.

Considerations such as these led us to the necessity of using a method which would enable the quantitative estimation of the regional vasomotor activity from the resistance shown by the vessels to the flow of blood [7]. The principle of this method, which we called the method of "resistography,"* consists of stabilization of the rate of the blood flow in the organ under study by means of a special perfusion pump [8].

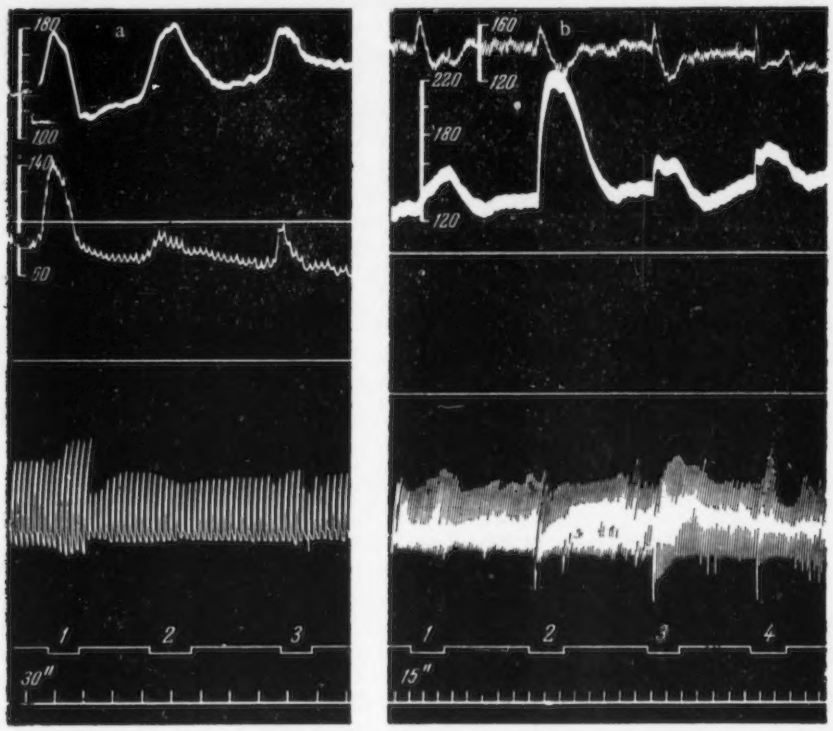

Fig. 1. Systemic and regional reflexes (a – on the walls of the branches of the superior mesenteric artery, b – on the renal vessels) during stimulation of mechanoreceptors: 1) of the carotid sinus; 2) of the large intestine (distension with air at a pressure of 100 mm mercury); 3) of the tibial nerve (intensity: a = 6v; b = 4v); 4) of the mechanoreceptors of the urinary bladder (pressure 60 mm mercury). Significance of the curves (from above downwards): a) perfusion pressure; its zero line, general arterial pressure (mercury manometer), its zero line, respiration, stimulation marker, time marker (30 seconds); b) general arterial pressure, perfusion pressure, zero lines (remainder as in tracing a), time marker (15 seconds).

By drawing blood from the proximal end of the artery supplying the organ and injecting it into the distal end of the artery, i.e. by carrying out autoperfusion, the pump maintains the minute volume of blood flow at a constant level. The perfusion pressure, recorded by a mercury manometer in the outlet of the pump, is in this case a function of the resistance of the vascular system of the organ, which is mainly determined by the size of the lumen of the arterioles, and consequently by the degree of vasomotor activity. Thanks to the constancy of the blood flow, a diminution in the lumen of the arterioles (increase in the resistance of the vessels) leads to a rise in the perfusion pressure, and dilatation of the arterioles (diminution of resistance) is accompanied by a fall in the perfusion pressure.

*We propose this term by analogy with the terms "plethysmography" and "rheography."

Experiments were carried out on cats under general anesthesia (urethane – 0.5 g/kg and chloralose – 0.03 g/kg). The arterial pressure (in the carotid artery) and the respiration were recorded in the usual way. In the first series of experiments using the method of resistography the resistance of the vessels in a loop of small intestine was measured. In order to exclude any collateral circulation the loop of small intestine was isolated from neighboring areas and the anastomosing branches of the superior mesenteric artery were ligated. In the second series of experiments measurements were made of the resistance of the vessels of the left or right kidney by autoperfusion of the vascular system of the organ through the renal artery. Heparin was used as an anticoagulant (given intravenously in a dose of 7.5 – 12.5 mg/kg).

In both series of experiments the mechanoreceptors of the large intestine and the urinary bladder were stimulated by distension of the organs with air, and the receptor zone of the carotid sinus (by compression of the carotid artery). The central end of the tibial nerve was stimulated by an alternating current with a frequency of 50 cps, the tension at the electrodes being controlled by a voltmeter. In the first series of experiments the mechanoreceptors of a loop of small intestine, proximal to the perfused area, was also stimulated. The stimulation of all these zones and nerve was repeated 3-4 times in the experiment, after which the vagus nerves on both sides were divided in the neck, and further stimulation applied. The duration of these last stimuli was usually 30 or 60 seconds. A strength of stimulation was selected which, as a rule, was adequate for producing perceptible reflexes affecting the arterial pressure.

EXPERIMENTAL RESULTS

As can be seen in Fig. 1, a, stimulation of the receptors of the carotid sinus and large intestine and of the afferent fibers of the tibial nerve causes both systemic and regional vasomotor reflexes which are registered in the superior mesenteric artery. It is characteristic, however, that the more powerful systemic reflexes from the carotid sinus and the tibial nerve (64 and 32 mm mercury) are accompanied by less pronounced regional reflexes (43 and 35 mm mercury). Meanwhile during stimulation of the mechanoreceptors of the large intestine, causing the smallest systemic reflex (22 mm of mercury), the largest regional reflex is produced (60 mm mercury).

Thus the degree of the reflex effects of individual interoceptive zones on the vessels of that particular region is not the same. Can this be connected with differences in the strength of stimulation? Direct comparison of the physical force of stimulation of different receptor zones, caused by different methods (distension of the walls of hollow organs, compression of the carotid artery, electrical stimulation of afferent fibers) is impossible; at the same time the effective strength of the stimulus may be estimated by the magnitude of the systemic reflex on the arterial pressure.

Magnitude of the Regional Reflexes in % of the Value of the Systemic Reflexes, Taken as 100% (mean results of all experiments)

Receptor zone	Vessels			
	of the kidney		of the superior mesenteric	
	%	number of observations	%	number of observations
Carotid sinus	49	16	53	18
Tibial nerve	51	14	66	24
Urinary bladder	121	17	130	9
Large intestine	272	6	178	25
Small intestine	–	–	350	13

Taking the magnitude of each systemic reflex as 100%, the magnitude of the corresponding regional reflex can be related to it (measured under conditions of resistography, in millimeters of mercury). In this case the regional reflex from the carotid sinus is found to be 67% of the magnitude of the systemic reflex appearing at the same time, and the regional reflex from the tibial nerve is 109% of the magnitude of the corresponding systemic reflex. The magnitude of the regional reflex from the receptors of the large intestine reaches a value of 252% of its corresponding systemic reflex.

The accuracy of this method is confirmed by Fig. 1,b, in which are shown tracings of the systemic and regional reflexes on the vessels of the kidney. In this experiment the differences in the power of the effects of the different interoceptive zones are shown particularly clearly, since in this particular animal the systemic reflexes from all four zones were almost equal. The maximum effect on the renal vessels arises from the receptors of the large intestine, reaching 100 mm mercury, and is 500% of the magnitude of the pressor phase of the systemic reflex. Corresponding values for other zones are as follows: carotid sinus – 15%, tibial nerve – 150% and urinary bladder – 123%.

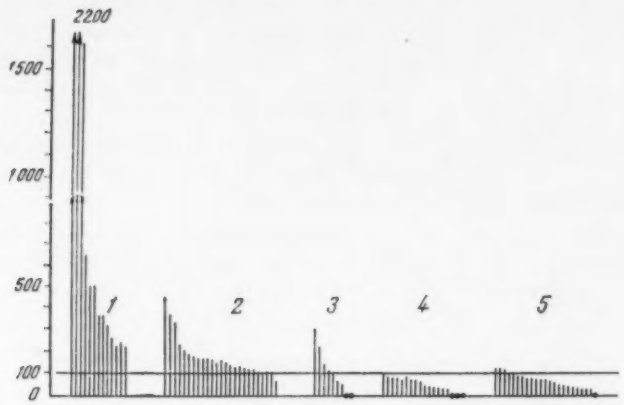

Fig. 2. The percentage relationship between the values of the systemic and regional reflexes on the vessels of the area supplied by the superior mesenteric artery (along the ordinate axis). The magnitude of the systemic reflex in each individual observation is taken as 100%. Along the axis of the abscissae – groups of reflexes resulting from stimulation of: 1) an area of the small intestine, 2) of large intestine, 3) urinary bladder, 4) carotid sinus, 5) tibial nerve. Results are from different experiments. Points along the axis of the abscissae – regional reflex absent.

The perfect regularity of the relationships between the systemic and regional reflexes for each interoceptive zone is shown convincingly by the curves which summarize the results of all the experiments of both series (Figs. 2 and 3) and the mean results which are shown in the Table.

The characteristic relationships between the systemic and regional reflexes for particular interoceptive zones are preserved also after bilateral division of the vagus nerves in the neck.*

It has to be pointed out that the regional reflexes recorded in the superior mesenteric artery arise as a result of constriction of the vessels of the small intestine and the portal vessels of the liver. In 2 experiments we measured the resistance of the vessels of the intestinal loop alone, by connecting a suitable branch of the mesenteric vein with a chlorovinyl tube to the external jugular vein. This naturally affected the absolute value of the regional reflexes, and correspondingly, their relationship to the systemic reflexes. However these relationships for each individual reflexogenic zone maintained the same order which corresponds to the mean results given in the Table.

Thus stimulation of different interoceptive zones, because of their dissimilar connections with the vessels of different organs, must lead to the appearance of unequal complexes of regional reflexes. The more or less

*This operation was carried out in order to minimize the possibility of an effect by the cardiac component on the magnitude of the systemic reflex. Simultaneous exclusion of the influence of the aortic reflexogenic zone slightly altered the absolute values of the systemic and regional reflexes, but did not noticeably affect the relationship between these values.

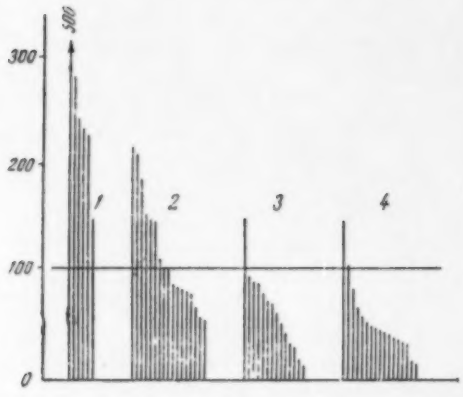

Fig. 3. Percentage relationship between the values of the systemic and regional reflexes on the vessels of the kidney (along the ordinate axis). The magnitude of the systemic reflex in each individual observation is taken as 100%. Along the axis of the abscissae – groups of reflexes resulting from stimulation of: 1) large intestine 2) urinary bladder, 3) tibial nerve, 4) cartoid sinus.

constant ratio between the values of the systemic and regional reflexes in different animals demonstrates the considerable degree of constancy of the complex generated by afferent signals from a particular zone.

It is known that stimulation of afferent fibers of different nerves in the limb excites a different number of motor neurones from the sum total making up the motor nucleus of the particular skeletal muscle (the principle of fractionation [3]). The facts described suggest that this principle is applicable to the vasomotor reflex, thereby indicating the existence of specific nuclei responsible for reflex control of the vessels of the various organs. At the present time it is not possible to state definitely whether these nuclei correspond to spinal preganglionic neurones or whether they are found in the bulbar vasomotor center. However it is clear that fractionation of vasomotor reflexes, with a different degree of connection between the interoceptive zones and the vessels of the individual organs, suggests some limitation on the spread of excitation, characteristic for each interoceptive zone, in the centers of these reflexes.

To counter the spatial conception of Cannon of the unrestricted irradiation of excitation in the sympathetic nervous system, Schweitzer [10] and Hellhorn [2] put forward the hypothesis of a more selective spread of excitation in its central divisions, suggesting that this was associated with differences in "synaptic resistance." The results of previous investigations [4-6] and also a number of new findings which we shall report in later articles, show that limitation of irradiation of excitation is determined by the simultaneous appearance of processes of inhibition and that limitation of irradiation of the reflexes has both a morphological and a functional basis.

SUMMARY

The author investigated the relationship of reflexes on arterial pressure (systemic) and regional reflexes on the blood vessels of kidneys and a portion of small intestine caused by stimulation of mechanoceptors of the urinary bladder, large and small intestine, carotid sinus and the tibial nerve. The regional reflexes were registered by the method of resistography (autoperfusion with stabilized minute blood volume). A definite relationship exists between the values of systemic and regional reflexes elicited from each receptor zone. These data show the existence of definite complexes of regional vasomotor reflexes caused by stimulation of the given interoceptive zone and point out the fractionation of reflexes in the centers of vasomotor regulation.

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REFLEX CHANGES IN THE ACTIVITY OF THE CARDIOVASCULAR SYSTEM PRODUCED BY CHEMICAL STIMULATION OF PERICARDIAL RECEPTORS

COMMUNICATION II. ON THE EFFECT OF COMPLETE OR PARTIAL EXCLUSION OF PERICARDIAL RECEPTORS ON CIRCULATION AND RESPIRATION

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It is known that stimulation of pericardial receptors in rabbits with nicotine has a depressor effect [6, 7], whereas exclusion of these receptors with novocain – a stable pressor effect on the blood pressure [6, 7, 11]. In a previous communication [3] we showed that both pressor and depressor reactions can be produced by chemical stimulation of pericardial receptors in cats. In some experiments only pressor and in others only depressor reactions were observed. However, in the majority of the animals it was possible to produce both reactions; pressor reactions were produced by relatively weak concentrations of nicotine and depressor reactions by higher concentrations. We expressed our conjecture that the character of the reaction of the cardiovascular system to stimulation of pericardial receptors is determined not by the specificity of different receptors conducting pressor or depressor effects, but by the number of receptors all of which are similar in so far as the effect on the cardiovascular system is concerned.

The purpose of this investigation was to determine which reactions of the cardiovascular and respiratory systems in cats are produced by excluding the pericardial receptors and how the reactions are influenced by stimulation of these receptors after their partial exclusion with small doses of novocain.

EXPERIMENTAL METHOD

Fundamental steps of the method are described in a previous communication [3]. Substances used for exclusion of receptors were dissolved in a Ringer-Locke solution (novocain – up to 1 or 2%, acetic acid – up to 3% concentration) and injected intrapericardially.

EXPERIMENTAL RESULTS

Injection of 1 – 2 ml of 2% novocain into the pericardial cavity usually excludes the pericardial receptors completely and, regardless of the character of resulting reactions, produces a rise in blood pressure and in cardiac and respiratory rate. It was found that in a number of instances stimulation and exclusion of receptors produced identical reactions by the cardiovascular system. Identical changes in the respiratory rate were produced in all experiments by both stimulation and exclusion of pericardial receptors.

In Fig. 1 is shown a kymogram in an experiment on a cat which reacted with a rise in blood pressure and in cardiac and respiratory rate to any, even the strongest (Fig. 1, a), chemical stimulation of pericardial receptors. Treatment of the pericardium with novocain caused similar, but somewhat less marked, changes following which the introduction of nicotine in the same concentration as before produced no effect. From

the point of view of current understanding this result can be explained only by assuming that nicotine stimulated certain – in this case pressor – receptors, and novocain excluded receptors sending tone-depressing impulses.

It is known that stimulation of chemoreceptors of the reflexogenic zone of the carotid sinus results in a rise of blood pressure, whereas increased stimulation of mechanoreceptors of this zone produces a reflex fall in general blood pressure. It was shown [12] that certain chemicals can stimulate not only chemo- but also mechanoreceptors in the zone of the carotid sinus. By analogy one may assume that in our experiments small doses of nicotine stimulated only the chemoreceptors, but larger doses stimulated the pericardial mechanoreceptors too (or increased their excitability) inasmuch as impulses from mechanoreceptors cause a fall in blood pressure [1, 5, 10]. If one were also to assume that the reflex tonus of pericardial mechanoreceptors is higher than that of chemoreceptors, it would be possible to explain the rise in blood pressure and in the cardiac and respiratory rates resulting from the treatment of the pericardium with novocain as due to exclusion of mechanoreceptors, and the origin of analogous effects resulting from treatment of the pericardium with nicotine as due to stimulation of chemoreceptors. In order to verify this assumption we selected E. Gernand's [9] method of selective exclusion of chemoreceptors with 0.5 M acetic acid.

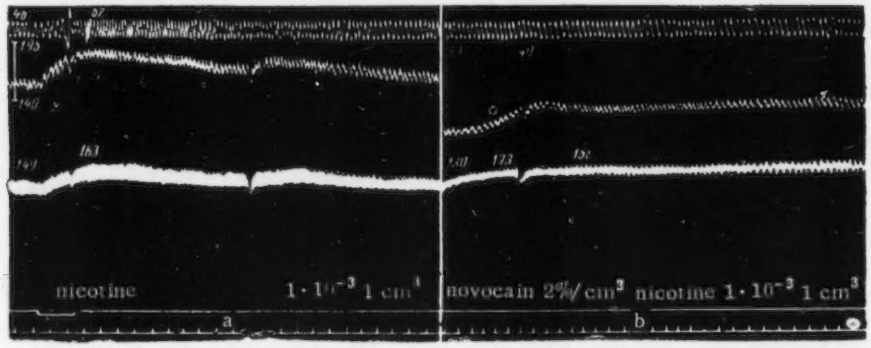

Fig. 1. Rise in blood pressure produced by stimulation of pericardial receptors with nicotine (a) and by exclusion with novocain (b). Meaning of tracings (from above down): respiration, blood pressure (mercury manometer), blood pressure (membranous manometer), record of stimulation, time record (2 seconds). Numerals along the respiration and blood pressure (membranous manometer) tracings indicate the respiration and pulse rates per minute.

It was found that treatment of the pericardium with one ml of 3% (0.5 M) acetic acid excludes irreversibly the possibility of reflex reactions to chemical stimulation of pericardial receptors and produces a lasting rise in blood pressure and in cardiac and respiratory rates strongly resembling the reaction to exclusion of pericardial receptors with novocain (compare Fig. 2,a and b; or Fig. 3). The difference is that the action of 3% acetic acid is manifested in a steeper growth of the reaction (Fig. 2,a) or in a greater intensity of the reaction in its initial stage (Fig. 3, e). The subsequent course of the reaction to acetic acid fully coincides with the reaction to novocain. Differences in the initial stages of the reactions can be explained as due to the effect of the brief but intense outburst of impulse which was observed consistently by E. Gernand during the first instants of the action of 3% acetic acid and before the exclusion of chemoreceptors. In our experiments, as in those of E. Gernand, mechanoreceptors preserved their excitability; this manifested itself in a momentary fall in blood pressure in response to rapid injection into the pericardial cavity of 10 ml of Ringer-Locke solution. Such effects were invariably observed when the stimulant was washed away (see Fig. 3, e).

Therefore, selective exclusion of pericardial chemoreceptors also produces strong rise in blood pressure and in cardiac and respiratory rate. This was observed in experiments on cats with various reactions of the cardiovascular system to chemical stimulation. If pressor reactions are linked with chemoreceptors, one could logically expect that their selective exclusion, at least in those experiments in which chemical stimulation always caused pressor reactions, must result in fall in blood pressure and in bradycardia.

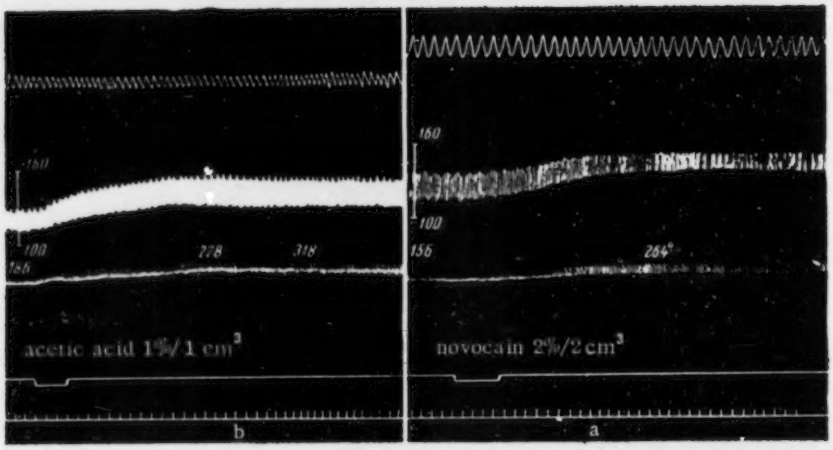

Fig. 2. Rise in blood pressure produced by exclusion of chemoreceptors (a) or all receptors (b) of the pericardium. Designations same as in Fig. 1.

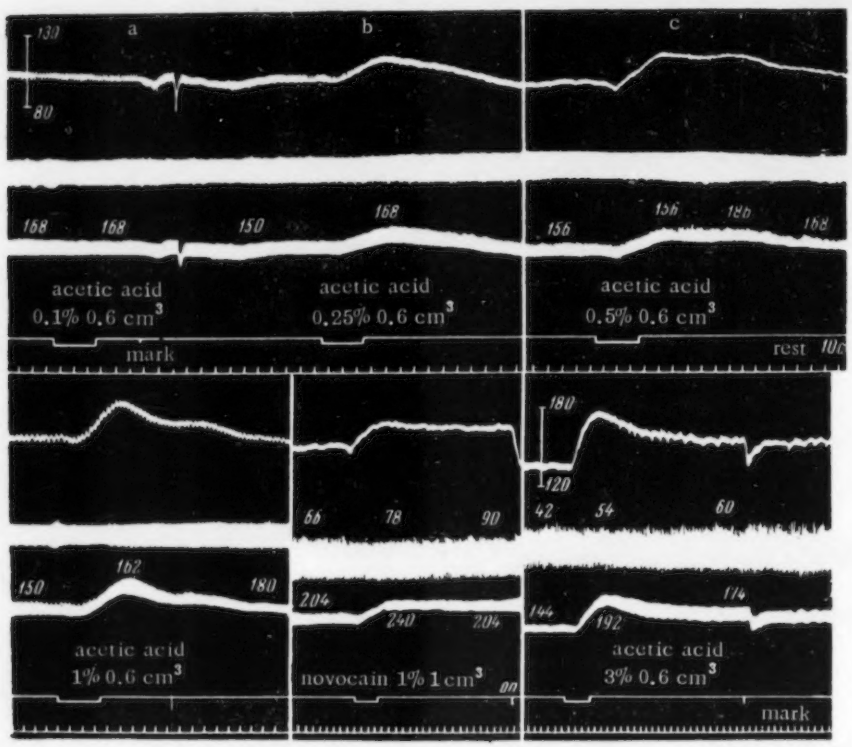

Fig. 3. Pressor reactions of blood pressure produced by treating the pericardium with acetic acid and with novocain. Meaning of tracings (from above down): blood pressure (mercury manometer), respiration, blood pressure (membranous manometer). The remaining designations same as in Fig. 1.

Treatment of the pericardium with acetic acid of lesser concentration (0.1 – 1%) reveals distinctly its stimulating action while its paralyzing action weakens and becomes reversible; however, it always manifests

itself, sharply limiting the duration of the impulse caused by the initial stimulation as a result of which pressor reactions accompanied by tachycardia are observed almost always in response to increasing concentrations of acetic acid (see Fig. 3). In these instances the effect of weak stimulation combined with subsequent effect of brief exclusion of receptors cannot produce a depressor reaction and bradycardia.

Subsequent experiments showed that treatment of the pericardium with novocain in amounts insufficient to completely exclude pericardial receptors but able to cause lowering of sensitivity of this reflexogenic zone, usually results in distinct rise of blood pressure and of cardiac and respiratory rate.

Under similar conditions, treatment of the pericardium with nicotine in low concentrations produces no effect on circulation and respiration. The effect produced by larger doses of nicotine was found to be weaker and similar to effects arising in response to small doses of nicotine prior to treatment of the pericardium with novocain. In experiments in which chemical stimulation of pericardial receptors produced only pressor or only depressor reactions, partial exclusion of these receptors with novocain was followed by weakening in intensity of the reactions to strong stimulation and by preservation of their original pressor or depressor character. In those experiments in which small doses of nicotine produced pressor reactions and tachycardia, and larger — depressor reactions and bradycardia, following treatment of receptors with a small dose of novocain large concentrations of nicotine produced not depressor reactions as prior to treatment with novocain, but pressor reactions, i.e. the same reaction as that produced in response to weak stimulation prior to treatment with novocain. Thus, by either decreasing the concentration of the stimulant or by preserving it but decreaing the sensitivity of the reflexogenic zone, we can convert the reaction of the cardiovascular system to chemical stimulation of pericardial receptors from depressor to pressor.

The facts described do not permit the consideration of the reflex rise in blood pressure and in cardiac and respiratory rates, resulting from the effect of novocain on the pericardium of cats, as a consequence of the exclusion of receptors which have a specific depressor effect on blood pressure, and thus cast doubt on the classification of receptors as pressor and depressor.

The assumption that the ability to send pressor effects is the function of chemoreceptors and the ability to send depressor effects produced by large concentrations of nicotine is the function of mechanoreceptors, is ruled out by experiments involving selective exclusion of pericardial chemoreceptors with acetic acid (0.5 M solution), in which a rise in blood pressure rather than a fall is observed as in exclusion of all pericardial receptors with novocain.

Against ascribing to individual receptors the ability to determine the direction of regulatory displacements caused by their stimulation are the experiments with partial exclusion of pericardial receptors with small doses of novocain. In the experiments we performed on cats which reacted to all stimulations of pericardial receptors with only pressor or only depressor reactions, we are convinced that novocain weakens both to the same extent. For this reason attention is drawn to the results of such experiments on animals in which weak concentrations of nicotine produced pressor reactions and high concentrations — depressor reactions. The fact that, after lowering of sensitivity of the reflexogenic zone of the pericardium with novocain, those concentrations of nicotine which called forth a fall in blood pressure and bradycardia now produced a rise in blood pressure and tachycardia, definitely speaks in favor of the assumption that impulses of individual receptors do not carry specifically pressor or depressor signals, but having arisen from a stimulation of a given intensity they determine the one or the other reaction on the part of the cardiovascular system. From experiments in which low concentrations of acetic acid were used, it becomes apparent that the duration of the volley of impulses which must be sufficiently great to give rise to a depressor reaction is an important characteristic of the signal significance of impulses from pericardial receptors. Analysis in receptors is limited, apparently, by differences in intensity and duration of the stimuli and by their ability to involve in the reaction of excitation a larger or smaller number of receptors; at the same time the character of efferent effects is determined by changes in the condition of nerve centers depending on the quantity of temporary and spatial distribution of afferent impulses entering the nerve centers [2].

Similarity of reactions to stimulation and exclusion of pericardial receptors can apparently be understood in the light of V. N. Chernigovskii's presentations [7, 8] that interoceptive impulses not only bring about these or those reflex reactions but also participate in formation of tonus in nerve centers, and not only those centers in which the reflex arc of their own reflexes is closed, but also in adjacent centers which effect the

vascular tonus in other areas [4], the heart rate, the respiratory rate, etc., i.e. those centers which, according to the type of combined reflexes [8], affect the vasomotor center as a whole and certain adjacent centers. Therefore, a rise in blood pressure and in cardiac and respiratory rates takes place when the total number of impulses entering the vasomotor center from the receptors of the pericardium and the reflexogenic zones of the carotid sinus, aorta and lungs is decreased.

SUMMARY

Exclusion of all pericardial receptors by novocain or only of its chemoreceptors by 0.5 M acetic acid brings about the rise of the blood pressure and increases the frequency of the heart beat in all cats. This takes place irrespective of whether the stimulation of these receptors by nicotine caused the rise of the blood pressure and tachycardia, depression and bradycardia, or (depending on the strength of stimulation) both reactions. Exclusion of the pericardial receptors, as well as their chemical stimulation, resulted in the increased frequency of respiration. After decrease of the sensitivity of the pericardial reflexogenic zone by small doses of novocain, reactions to small doses of nicotine disappeared, while those to large ones became weaker in animals in which all doses of nicotine cuased only pressor or only depressor reactions. In animals in which weak stimulation brought about a pressor reaction and strong stimulation a depressor one, pressor reactions resulted after strong stimulation following decrease of the sensitivity of receptors by novocain.

The conclusion is drawn that reflex reactions caused by exclusion of pericardial receptors cannot be explained by exclusion of afferent tone depressing impulses.

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THE EFFECT OF INSULIN HYPOGLYCEMIA ON THE CONTRACTION OF THE NICTITATING MEMBRANE OF THE CAT DURING STIMULATION OF PREGANGLIONIC SYMPATHETIC NERVE FIBERS

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The working of the nervous system, and in particular of its autonomic division, at different levels of the blood sugar is one of great interest and a large number of investigations have been devoted to it (see the summary by Hellhorn [12]. In recent years several investigations on this subject have been carried out in our own laboratory [1-7]. The majority of workers point out that during slight hypoglycemia excitation of the sympathetic division of the nervous system predominates, but as hypoglycemia increases the parasympathetic division becomes more excited. However there is an almost complete absence of a differential analysis of the part played in these reactions by the cells of the various levels of the sympathetic division; in particular little work has been done on the study of the function of the sympathetic ganglia during hypoglycemia.

McIntosh [14] discovered that during perfusion of the superior cervical ganglion of the cat with Ringer's solution not containing glucose, stimulation of the preganglionic nerve prevents the transmission of impulses (as shown by diminution of the contraction of the nictitating membrane) and causes a fall in the acetylcholine content; during perfusion with the same solution containing glucose, according to Brown and Feldberg [11] even prolonged stimulation causes no change in the acetylcholine content of the ganglion tissue.

Kahlson and McIntosh [13] showed that during perfusion of the superior cervical ganglion with a solution not containing glucose, with prolonged stimulation a more rapid suppression of the transmission process appears (tested by the contraction of the nictitating membrane) than when glucose is present in the perfusion fluid; however the reaction of the ganglion to actylcholine injected from outside is not affected. Perry and Reinert [15] found that there is a falling off in the reaction to injected acetylcholine.

Conditions of perfusion and total deprivation of glucose are far from natural; for this reason it is of interest to investigate the function of the superior cervical ganglion in the intact animal in a state of insulin hypoglycemia.

The aim of this investigation was to trace the changes during insulin hypoglycemia of the threshold of excitation of the preganglionic nerve fibers and also the transmission of submaximal stimuli. The indicator used was the contraction of the nictitating membrane of the cat.

EXPERIMENTAL METHOD

Experiments were carried out on cats which were starved for 24 hours. The first group of experiments was performed under chloralose anesthesia. Ringer's solution, containing 0.8% chloralose, was injected (after induction with ether) into the femoral vein in a dose of 100 mg of anesthetic per kg body weight of the animal. The cervical sympathetic nerve was dissected out, ligated and divided; the peripheral end was laid on

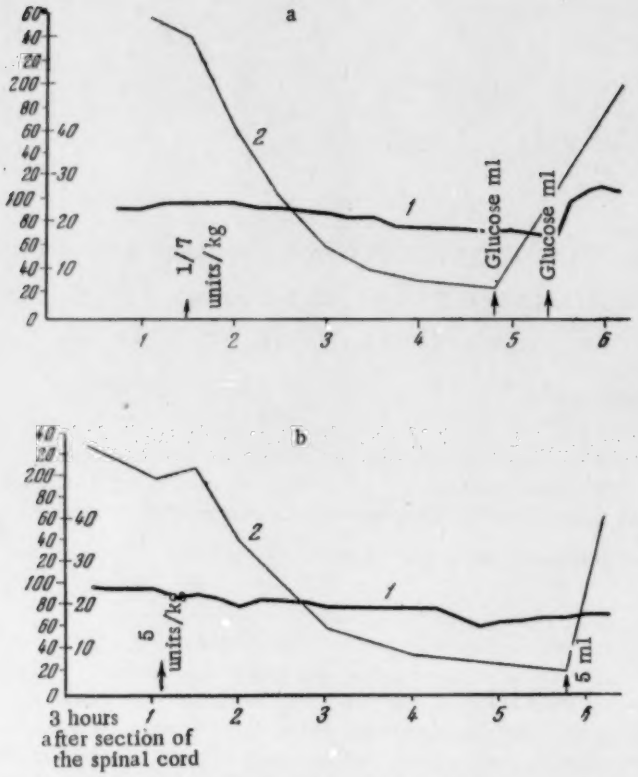

Fig. 1. The effect of insulin hypoglycemia on the threshold of stimulation of the preganglionic cervical sympathetic nerve, as indicated by contraction of the nictitating membrane of the cat. a) During chloralose anesthesia (experiment on January 15, 1957), b) during section of the spinal cord (experiment on April 13, 1957); 1) value of the threshold of stimulation; 2) level of the blood sugar in mg %. Along the axis of the abscissae – time in hours; along the ordinate axis: figures on the left – blood sugar level in mg %, figures on the right – value of the threshold of stimulation in divisions of the potentiometer scale. The first arrow indicates the moment of injection of insulin, the second and third – the moments of injection of glucose.

immersible electrodes. Gauze drainage strips were placed in the wound, soaked in Ringer's solution. To prevent displacement, the electrodes were fixed with a clamp attached to the stand. As a result of many hours of exposure on the electrodes, despite great care in some of the experiments the nerves were nevertheless damaged; these experiments were not considered. The nictitating membranes were grasped in clips and their contractions recorded on the kymograph by means of Swedish levers. The animal was kept warm throughout the experiment. When preparations were completed an interval of 1-2 hours was allowed, and then measurment of the thresholds of stimulation of the nerve was begun. Stimulation was carried out by means of an induction coil with an alternating current of a frequency of 50 cps. Into the secondary circuit of the coil was introduced a potentiometer with a scale graduated in 100 divisions. In the records and on the graphs the voltage of the stimulating current is expressed not in absolute values but in divisions of the potentiometer scale. When the threshold was being determined the stimulus lasted 3 seconds, with an interval of not less than 3 minutes between stimuli. The submaximal stimulation lasted 15 seconds and was given not more often than once in 15 minutes. In all the experiments blood was taken at definite intervals for estimation of the blood sugar content by the Hagedorn-Jensen method. After the threshold of stimulation of the nerve had remained

constant for not less than an hour, insulin in a dose of 5 units per 1 kg body weight of the animal was injected into the femoral vein.

In the second group of experiments, after ether induction the spinal cord was divided below the medulla; no other conditions were altered.

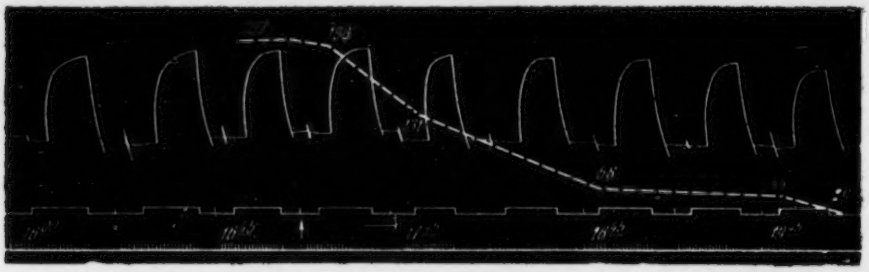

Fig. 2. Contraction of the nictitating membrane of the cat during submaximal stimulation of the preganglionic nerve during a state of insulin hypoglycemia (experiment on April 19, 1957). Significance of the curves: continuous lines (from above downwards) – record of the contraction of the nictitating membrane, marker of stimulation of the nerve (a rise in the line), time marker (1 second); broken line – curve of the blood sugar level in mg % (the zero line for measurement is the line of the time marker). The arrow indicates the moment of injection of insulin (5 units/kg).

In the third group of experiments, in order to ascertain the influence of hypoglycemia on the nictitating membrane, stimulation of the sympathetic nerve was replaced by intravenous injection of adrenalin. In order to increase the sensitivity of the nictitating membrane to adrenalin the right superior cervical ganglion was excised from 3-8 days before the experiment. The doses of adrenalin in different experiments were altered in accordance with the individual susceptibility of the animal (from 0.5 ml of a 1:20,000 solution to 1 ml of a 1:10,000 solution).

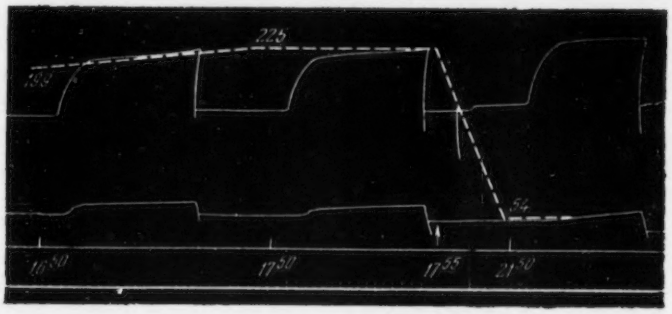

Fig. 3. The effect of insulin hypoglycemia on the contraction of the nictitating membrane of the cat under the influence of injection of adrenalin (experiment on May 21, 1957). Significance of the curves: continuous lines (from above downwards) – contraction of the right nictitating membrane (denervated), contraction of the left nictitating membrane (normal), marker of injection of adrenalin (0.5 ml of 0.02%), time marker (5 seconds); broken line – curve of the blood sugar level in mg %. The arrow indicates the moment of injection of insulin (5 units/kg).

EXPERIMENTAL RESULTS

The effect of insulin hypoglycemia on the threshold of stimulation of the preganglionic nerve fibers, recorded by the contraction of the nictitating membrane, was studied in 17 experiments (12 experiments under chloralose anesthesia and 5 experiments with section of the spinal cord).

In 8 of the experiments under chloralose anesthesia and in all the experiments in which the spinal cord was divided, during severe changes in the level of the blood sugar (from 250 to 20 mg%) no changes in the threshold of stimulation were observed, as may be seen in Fig. 1, a and b). In 3 experiments under chloralose anesthesia variations were noted in the threshold of stimulation, both a rise and fall. However, as shown by control experiments, in both the anesthetized and the spinal animals with a relatively stable high level of the blood sugar even more significant variations in the threshold are possible; only in one experiment under chloralose anesthesia did a slight hypoglycemia (61 mg %) give rise to an increase in the threshold of stimulation, but injection of glucose restored it to normal.

In some experiments in addition to determination of the threshold the contraction of the nictitating membrane was recorded in response to a submaximal stimulus (1.5 – 2 rheobases). In these conditions also it was not possible to detect any changes in the function of the ganglion during hypoglycemia (see Fig. 2).

The experiments with injection of adrenalin showed that insulin hypoglycemia does not cause changes in the reaction of the nictitating membrane to injection of adrenalin. The variations observed in the magnitude of the contractions were no greater than the same variations with the blood sugar at a high level (Fig. 3).

In the conditions which we adopted a fall in the level of the blood sugar caused no detectable effect on the function of the superior cervical ganglion.

Hellhorn [12] points out the significantly greater resistence to hypoglycemia of the sympathetic nerve cells as compared with the somatic. He considers this to be the result of the low metabolism of the sympathetic cells. Grounds in favor of this explanation in respect of the superior cervical ganglion may be found in the work by Bargeton [9], who showed that the ganglion functions even as long as 10-15 minutes after total cessation of its blood supply, and by Bronk [10], who found that even after anemia lasting 6 hours restoration of the blood flow leads to recovery of the function of the ganglion.

In the investigations of McIntosh [14], Kahlson and McIntosh [13], Perry and Reinert [15] in the first place the ganglion was completely deprived of glucose, which does not happed during a hypoglycemic state in the intact animal. Kahlson and McIntosh point out even negligible doses of glucose (starting with 5.6 μ g) restore the function of the ganglion. In the second place these authors cited used stimuli which were prolonged (for several hours), exhausting and supermaximal in the strength. Kahlson and McIntosh found that the effect of depriving the ganglion of glucose begins to show itself only after 10-15 minutes of maximum stimulation and that short interruptions lead to the restoration of transmission, but with short (1-2 minutes) periodic stimuli even complete exclusion of glucose from the perfusion fluid does not affect the magnitude of the contraction of the nictitating membrane. In the third place the investigations cited were carried out by perfusion of the ganglion. In these circumstances the humoral influence of the animal on the ganglion is excluded and the blood is replaced by saline solution. Perry and Reinert [15] point out that glutamate, for example, normalized the abnormal action of hexamethonium on the denervated ganglion only if it was given at the very beginning of perfusion. After perfusion had proceeded for a few minutes the effect of glutamate was lost. These authors refer to the work of Raaflaub [16] who showed that in a saline medium it is possible for enzymes to be washed out of the mitochondria, and consequently for severe disturbances of metabolism to take place.

Thus our findings do not contradict those of the authors cited, although in the latter case the changes in the function of the superior cervical ganglion were observed only in experimental conditions far removed from natural ones.

A. L. Shabadash [8], using a method similar to our own, found that glucose (especially glucose with insulin) suppresses the process of transmission in the ganglion, and accounts for this effect by overfilling of the preganglionic end plates with glycogen. We never observed suppression of this sort. This contradiction may possibly be explained by the fact that A. L. Shabadash obtained his results during mixed barbiturate anesthesia, and barbiturates are known to act on the endings of sympathetic nerve fibers. In two of our own experiments the use of pentothal sodium as an anesthetic caused a sharp reduction in the contraction of the nictitating membrane, in consequence of which we no longer used this anesthetic.

Our results demonstrate that during insulin hypoglycemia, under acute experimental conditions, the threshold of stimulation of the preganglionic nerve fibers leading to the superior cervical ganglion of the cat, as shown by contraction of the nictitating membrane, showed no appreciable change; contraction of the nictitating membrane during subminimal stimulation of the preganglionic nerve fibers and also under the influence of injection of adrenalin is also unaffected.

SUMMARY

Acute experiments were performed on cats with insulin hypoglycemia. It was established that the threshold of stimulation of preganglionic nerve fibers passing to the superior cervical ganglion (tested by the contraction of the nictitating membrane) does not show any significant change.

The contraction of the nictitating membrane remains unchanged in submaximal stimulation of the preganglionic nerve fibers. Adrenalin injection also has no effect on its contraction.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE STUDY OF BACTERIOSTATIC AND CHEMOTHERAPEUTIC ACTION OF THE ANTITUBERCULOUS PREPARATION 1314 AND ITS CHLORHYDRATE

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Dr. Libermann and his co-workers [2] in France synthesized in 1956 a new antituberculous preparation, which they named 1314.

Investigations carried out by N. Rist and F. Grunbuch [1] in the Pasteur Institute in Paris showed that this preparation is effective in the treatment of experimental tuberculosis.

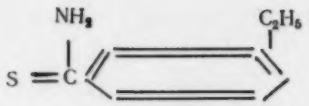
In view of the fact that the synthesis method of preparation 1314 suggested by the French investigators was found to be quite complicated, Prof. N. K. Kochetkov, N. F. Kucherovaia, R. M. Khomutov and E. I. Budovskii of the Division of Organic Synthesis of the Institute of Pharmacology and Chemotherapy AMN SSSR developed in 1957 a simpler and more convenient method for synthesizing this preparation.

Realizing the low solubility of preparation 1314 in water, a method for obtaining its hydrochloride also developed.

Preparation 1314 is thiamido-α-ethylisonicotinic acid.

The empiric formula is $C_8H_{10}N_2S$.

The structural formula is

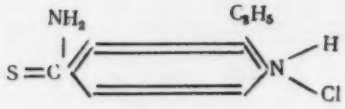


Molecular weight is 166.

The preparation is not water soluble.

The water soluble form of preparation 1314 is represented by the hydrochloride of thiamido-α-ethylisonicotinic acid. Its empirical formula is $C_8H_{10}N_2S \cdot HCl$.

The structural formula is



The molecular weight is 202.5.

In this communication we are presenting data on the study of bacteriostatic and chemotherapeutic action of preparation 1314.

EXPERIMENTAL METHOD AND RESULTS

In view of the fact that chemically the basic preparation 1314 and its hydrochloride are identical and taking advantage of the satisfactory solubility of the latter in water, we employed the hydrochloride of preparation 1314 in our in vitro experiments.

Antibacterial activity was studied in liquid bouillon medium to which the preparation was added in increasing concentrations beginning with 2 and ending with 200μ g/ml. The microbial component for all types of bacterial cultures consisted of 250,000 microbes per 1 ml of medium.

The results of the experiments are presented in Table 1.

TABLE 1

Activity of the Hydrochloride of Preparation 1314 Regarding Various Microbes

Culture	Activity in 200 g/ml
Staphyl. 209	None
Staphyl. Wood	»
B. coli is	»
B. coli 35	»
B. coli resistant	»
Proteus Ox_{19}	»
B. pyocyaneum	»
Sarcina lutea	»
B. subtilis	»
B. mycoides P-20	»
B. typhi abd. 4446	»
B. paratyphi B 42	»
B. dys. Flexneri	»
B. paratyphi A 299	»
B. dys. Sonne 714	»

As shown in Table 1, preparation 1314 in concentration of 200 μg/ml possesses no antibacterial activity against both gram positive and gram negative microbes.

The action of hydrochloride of preparation 1314 on Mycobacterium tuberculosis was studied by us on Soton's synthetic liquid medium to which 10% of human plasma was added. The following tuberculous cultures were used in the experiment: laboratory culture type B. bovinus 8.4, and freshly isolated cultures of human types 123, 126, 122 and BCG strain.

Strain 126 was sensitive to streptomycin, phthivazide (isonicotinic acid hydrazide derivative) and PAS; strain 123 – resistant to 100 U streptomycin, 10 μg/ml phthivazide and 50 μg/ml PAS, strains 122 and 346 – resistant to 10μ g/ml phthivazide and sensitive to streptomycin and PAS. In the case of each strain investigated we ran three experiments with 27 ml of microbial component to one ml of the medium. The results were recorded every seven days. Chemotherapeutic activity was noted only in those cases where controls showed a good culture growth (+++).

Data presented in Table 2 is an average of three experiments.

As shown in Table 2, preparation 1314 possesses high bacteriostatic action (in concentrations of 0.5 to 0.75 μg/ml) on various types of Mycobacterium tuberculosis, which property brings it close to streptomycin from the chemotherapeutic standpoint.

Our attention was drawn to the fact that beginning with each third week after the initiation of the experiment a larger amount of preparation 1314 was required to maintain suppression of the growth of Mycobacterium tuberculosis. This phenomenon deserves special study.

Chemotherapeutic activity of the preparation was studied in white mice having hematogenous tuberculosis. White mice weighing 18-20 g were used. The animals were infected by the intravenous injection of 0.1 mg of B. bovinus 8 culture. Treatment was begun with preparation 1314 as well as its hydrochloride on the day following infection. Different groups of animals were given different doses and by different routes. In the controls and the treated groups there were 15 mice in each. The experimental results were evaluated by macroscopic changes in the lungs, the weight of the lungs and the number of tubercle bacilli in lung smears. All changes were recorded by the three-point system of M. V. Trius.

In Table 3 are presented average indices of macroscopic changes for each group of animals receiving preparation 1314.

As shown in Table 3, the preparation was found to be highly effective in the treatment of hematogenous tuberculosis in white mice. Indices of macroscopic changes in the lungs are low, the weight of the lungs as

TABLE 2

Action of Preparation 1314 on Mycobacterium tuberculosis

Name of strain	Record of results by days			
	7	14	21	28
	Activity in μg/ml			
B. bovinus 8	—	0, 5	1	5
Human type 123 . .	—	0,75	3,5	5
Same 123	—	0, 6	3,0	7,5
» » 122	—	0, 6	3,5	7,5
» » 346	—	0, 7	3,0	5,5
BCG	—	0, 6	2,0	6,0

TABLE 3

Index of Macroscopic Changes, Weight of Lungs and the Number of Tubercle Bacilli in Lungs Smears of Treated and Untreated (control) Mice

Animals	Daily dose of preparation (mg)	Method of administration	Average index of macroscopic lung changes	Average weight of lungs (mg)	Presence of tubercle bacilli in lung smears
Untreated (controls	—	—	2,66	598	+++
1st treated group . .	1	per os	0,66	216	0
2nd » » . .	2	» »	0,73	211	0
3rd » » . .	3	» »	0,23	200	0

TABLE 4

Index of Macroscopic Changes, Weight of Lungs and Number of Tubercle Bacilli in Lung Smears of Treated and Untreated (control) Mice

Animals	Daily dose of preparation (mg)	Method of administration	Average index of macroscopic lung changes	Average weight of lungs (mg)	Presence of tubercle bacilli in lung smears
Untreated (controls)	—	—	2,66	598	+++
1st treated group . .	1	per os	0, 9	221	0
2nd » »	2	» »	0,46	220	0
3rd » »	3	» »	0,33	218	0
4th » »	2	subcutaneous	0, 5	201	0
5th » »	3	» »	0,166	188	0

compared to that in healthy animals is unchanged, tubercle bacilli are absent in lung smears. However, when the dosage was 3 mg flavedo of the liver was observed at autopsy. For this reason under the conditions of the experiment doses of 1 and 2 mg per mouse must be used for therapy. Simultaneously and under the same conditions a chemotherapeutic study of the hydrochloride of preparation 1314 was conducted with administrations subcutaneously and orally. There were 15 mice in each treated and each control group. Average data on the

chemotherapeutic activity of the preparation is presented in Table 4. It must be pointed out that all treated animals survived the experiment whereas 9 of the 15 controls died between the 28th and 32nd day.

As Table 4 shows, the hydrochloride of preparation 1314 possesses high chemotherapeutic activity when administered subcutaneously and orally, which testifies to the identity of properties of preparation 1314 and its chlorhydrate.

Besides, when the preparation is administered subcutaneously in doses of 2 and 3 mg the indices of macroscopic changes in the lungs are low and equal to 0.5 and 0.166, the weight of the lungs is normal, and tubercle bacilli are absent in lung smears. An average weight gain of 2.5 g was observed in the animals during the experiment. Their survival throughout the experiment testifies to the effectiveness of the preparation.

When the preparation was administered subcutaneously in 3 mg doses some flaccidity and flavedo of the liver were found. Further investigations of chemotherapeutic properties of the preparation on animals of other species is in progress.

SUMMARY

The authors conducted an experimental study of the bacteriostatic and chemotherapeutic effect of the antituberculous preparation 1314 (thiamido-α-ethylisonicotinic acid) and its hydrochloride. The preparation appeared to be nonactive with respect to gram positive and gram negative microbes in vitro. However, it possesses a high bacteriostatic activity with respect to the Mycobacterium tuberculosis including certain strains resistant to other antibiotics. The chemotherapeutic effect of the preparation was checked on white mice with experimentally induced hematogenous tuberculosis. Daily administration of the preparations per os (1-2 mg per mouse) retards the development of tuberculosis. The same results were obtained with administration of the hydrochloride of the 1314 per os and subcutaneously in the dose of 2 mg per mouse.

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ELECTROPHYSIOLOGICAL ANALYSIS OF DISTURBANCES IN REFLEX ACTIVITY OF THE SPINAL CORD IN EXPERIMENTAL BOTULISM TYPE A IN WARM - AND COLD-BLOODED ANIMALS

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It was determined by us [2, 3, 4] in the investigation of the action of botulism toxin on the nervous system that exclusion of nervous influences on tissue results from the damaging effect of the toxin on afferent divisions of nerve centers. However, the mechanographic method used by us in published reports enabled us in the case of botulism to obtain only an incomplete idea of the actual functional disturbances in the nervous elements.

In this connection the problem of the current project was the study of electrical activity of the anterior roots of the spinal cord when stimulated reflexively in the presence of a unilateral involvement of cord segments produced by small doses of botulism toxin with resulting "local" form of botulism – paralysis of one of the hind legs. The second, unparalyzed, side served as a control. Aside from this, it was of interest to clarify the functional condition of the peripheral motor nerve conductors in the zone of botulism paralysis. In the role of indices characteristic of the functional condition of motor nerve trunks we used lability and duration of the phases of absolute and relative refractivity.

The literature indicates that the magnitude of the given indices reacts delicately to metabolic changes in nerve trunks [6].

In relation to the electrical activity of striated muscles and nerves, it was made clear that botulism toxin does not produce any changes in oscillograms of motor nerves and does not slow down transmission of excitation waves along them [7, 8, 9]. This was confirmed on the A, B, C fibers of the sciatic, phrenic and vagus nerves [7]. However, when neuromuscular block due to botulism develops, recording of the muscular electropotentials showed that when a motor nerve is stimulated by two maximal stimuli with an interval between of 10 to 300 milliseconds, the second stimulus causes a greater muscle activity current than the first. The authors explain such of fact by summation of excitations in an area located somewhere near myoneural junctions.

EXPERIMENTAL METHODS AND RESULTS

Experiments were carried out on primed and unprimed cats and frogs. Priming was produced by the administration of botulism toxin type A (1 Mld = 0.00005 mg), dissolved in saline solution, into the gastrocnemius muscle in the following doses: cats – 0.25 mg/kg body weight (5,000 mouse Mld , frogs – 0.15 – 0.2 mg per 35-40 gm body weight (3,000 – 4,000 mouse Mld). In cats receiving the indicated doses, "local" botulism developed 2-3 days later – a complete paralysis of the muscles of the involved hind leg. In frogs, however, "local" botulism also developed in 2-3 days, but by the 4th – 5th day it went on, as a rule, to a "generalized" form, i.e. general paralytic botulism syndrome. Recording of activity currents from the anterior roots of the spinal cord and investigation of lability and of refractivity phases were carried out in cases of "local" botulism on both sides – the paralyzed and, as a control, the unparalyzed, but in cases of "general" – only on one side.

Under deep chloroform-ether anesthesia the spinal canal was opened at the lumbar level and the anterior roots of the spinal cord at the level of the 6th and 7th lumbar segments were severed; the skull of the animal was then trephined for decerebration. Following decerebration the anesthesia was discontinued and nerve twigs to the gastrocnemius were severed. One to 2 hours after decerebration the central end of the divided anterior root of the 7th lumbar segment was placed on platinum electrodes connected with the entry to the amplifier and the central end of the divided muscle nerve twig placed on the electrodes connected with the exit of the generator of the universal apparatus constructed by us for electrophysiological investigations [5].

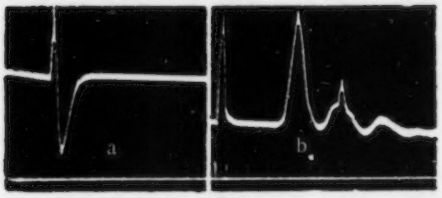

Fig. 1. Electrical activity of the anterior roots of the 7th lumbar segment of the spinal cord of a cat on the paralyzed (a) and contralateral side (b). Time mark — 1,000 cycles per second.

Mono- and polysynaptic and electrotonic potentials recorded in the anterior roots of the spinal cord were produced by stimulation of the central ends of the divided nerve twigs with rectangular stimuli of varying amplitude. Similar methods were often employed by domestic investigators [1]. Phases of relative and absolute refractivity were investigated by stimulating the trunk of the greater tibial and lesser tibial nerves with paired impulses with regulated intervals between them. The beginning of the phase of relative refractivity was recognized by the decrease of the second activity current on the oscillogram, the beginning of the phase of absolute refractivity — by the complete disappearance of the current. The beginning of transformation of the stimulation rhythm was recognized as the index of lability. Activity currents of the anterior roots of the spinal cord and of the motor nerves were photographed with a small camera ("Exacta," "Zenith") from the screen of the oscilloscope of the universal apparatus with momentaneous or repeated unwinding. In some of the experiments currents of nerve activity were recorded with the aid of a trained oscillograph of the type MPO-2.

Almost an analagous method was used in experiments on frogs. Under deep chloroform-ether anesthesia the spinal canal of the animals was opened and the anterior roots at the level of the 7th and 8th segments of the cord were severed. The latter were divided at the entrance to the intervertebral orifices. Anesthesia was discontinued and 2-3 hours after dissection the part of the experiment dealing with potentials of activity of the central ends of the divided anterior roots was carried out. Electrical activity of the root was provoked, as in the experiments with cats, by stimulation of the central ends of the divided nerve twigs to the gastrocnemius muscle of the ipsilateral side with single rectangular impulses. Investigation of the refractivity phases did not differ from analogous experiments on cats.

A total of 59 experiments was performed upon cats and 94 upon frogs.

In the first series of observations we investigated the character of electrical activity of the anterior roots of the primed segments of the spinal cord by stimulating the ipsilateral sensory nerve twigs of the completely paralyzed extremity. As controls we used analogous data obtained from the anterior roots of unprimed areas of spinal cord or from normal animals. Inasmuch as paralyses of the involved extremity develop only on the 2nd or 3rd day after intramuscular injection of botulism toxin (in sublethal doses), recording of electropotentials of the anterior roots was not done until after this period. In investigating the electropotentials of the anterior roots a total of 18 experiments were performed on cats (from the 4th to the 15th day after priming) and 14 similar experiments on frogs (from the 2nd to the 7th day after administration of the toxin). In all animals a form of "local" botulism developed — paralysis of the involved hind leg. Experiments showed that in warmblooded as well as in coldblooded animals once paralysis of an extremity develops it is impossible to record, during any of the indicated periods, any electrical activity in the anterior roots of the primed segments of the spinal cord; at the same time on the symmetrical unparalyzed side similar activity undergoes no noticeable changes.

Figure 1a shows that stimulation of the central end of a divided nerve twig to the gastrocnemius muscle of the paralyzed extremity with a single shock of a rectangular current on the 5th day after the administration of botulism toxin is not accompanied by the potential of activity in the anterior root of the primed segment of the spinal cord; all that is observed is the artefact of the loop of the stimulating current. In Fig. 1b is shown the control oscillogram of the current of activity in the anterior root of the spinal cord of the same animal, but on the unparalyzed side from which it is evident that development of mono- and polysynaptic potential in the anterior root is produced by stimulation of the sensory nerve twigs of the gastrocnemius muscle.

Similar fall of electrotonic currents of activity in the anterior roots was noted on the paralyzed side in frogs with "local" botulism; on the unparalyzed side the electrotonic reactions of the anterior roots of the spinal cord were preserved. It must be pointed out that the magnitude of the latent periods and the character of the electrical activity of the anterior roots on the unparalyzed side of animals with "local" botulism were found to be entirely similar to corresponding controls obtained in 2 experiments on normal cats and in 5 experiments on frogs, which in turn did not numerically differ from data to be found in the literature [1].

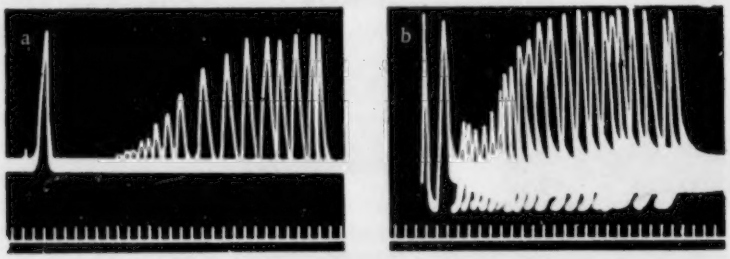

Fig. 2. Phases of refractivity of the lesser tibial nerves of the cat's paralyzed (a) and unparalyzed (b) extremity on the 22nd day after priming. Time mark – 1,000 cycles per second.

Thus, experimental results with "local" botulism clearly indicate that sausage poison causes the paralytic syndrome as a result of exclusion of motor neurons of the anterior roots of the spinal cord which blocks transmission of excitation to motor nerve conductors.

Further it became interesting to determine whether any change takes place in the functional properties of motor nerve trunks following the affection of their " trophic" centers – nerve cells – with botulism toxin. Data from the literature testifies to the fact that the oscillogram of a nerve primed with botulism toxin does not differ from that of a normal nerve; however, we were unable to find any information in the literature concerning changes in functional properties of nerve conductors in botulism. In this connection we investigated the duration of phases of absolute and relative refractivity at various intervals following the development of botulism paralysis, and in some experiments – immediately the lability of the greater or lesser tibial nerve in the zone of "local" botulism. As controls we used analogous indices obtained on nerves of the contralateral unparalyzed side or in experiments on normal animals.

A total of 34 experiments were carried out on cats (from the 3rd to the 25th day after priming) and 32 experiments on frogs (from the 2nd to the 7th day after administration of toxin).

Experiments on both species of animals revealed similar regularity: early after the development of the paralytic botulism syndrome (up to the 4th – 6th day in cats and up to the 2nd day in frogs) the duration of the refractivity phases and the lability of motor nerves remain close to normal. Thus, the duration of the phase of relative refractivity of the greater and lesser tibial nerves on the unparalyzed side in cats was 4 – 8 milliseconds and of absolute – 0.8 – 1.8 milliseconds. In the unparalyzed sciatic nerve of frogs the duration of the phase of relative refractivity was 12 – 16 milliseconds, and absolute – 2.2 – 3.2 milliseconds. Identical values were obtained in 5 experiments on normal cats and in 8 experiments on frogs. In the zone of botulism paralysis during the early periods following priming referred to above, the duration of the relative phase of refractivity of the motor nerves in cats was 9 – 13 milliseconds, and absolute 1.8 – 3.2 milliseconds; in frogs the first increased to 17, the second – to 3.7 – 5 milliseconds. The lability of the paralyzed nerves underwent a change too: in normal nerve, transformations of the excitation rhythm were noted at a frequency of approximately 500 cycles per second, but in the paralyzed nerve – at a frequency of 450 cycles per second and less.

Beginning with the 7th – 8th day after development of paralysis in cats and with the 3rd – 4th day in frogs, refractivity phases progressively continue to increase and reach the maximum: in cats – on the 10th – 14th day and in frogs on the 5th – 6th day. The phase magnitude remains relatively stable during the later stages of the investigation. During the current stage of intoxication the duration of the phase of relative refractivity of motor nerves in the cat is 18 – 22 milliseconds and of absolute refractivity – 6 – 12 milliseconds; in frogs the former reaches 28 – 32 milliseconds and the latter – 8 - 10 milliseconds.

In Fig. 2 a, one can see that there is a considerable increase in the phases of relative and absolute refractivity in the zone of "local" paralysis in cats following intramuscular injection of botulism toxin type A in the dose of 0.3 ml $1 \cdot 1^{-3}$ per 1 kg body weight into left hind leg. In Fig. 2 b is presented an oscillogram of the lesser tibial nerve on the unparalyzed side of the same animal. The duration of the refractivity phases is within normal limits. Thus, despite the ability of a motor nerve in the presence of botulism to conduct excitation, its functional properties are significantly altered. An important role in the dynamics of changes in functional properties of motor nerves in the form of substantial increase in refractivity phases and decrease in lability is played by the disturbance of trophic effects of spinal nerve centers whose activity is the first to be destroyed by botulism toxin. In this connection it can be assumed that alteration in the functional properties of motor nerve conductors is due to severe disturbances in their metabolism.

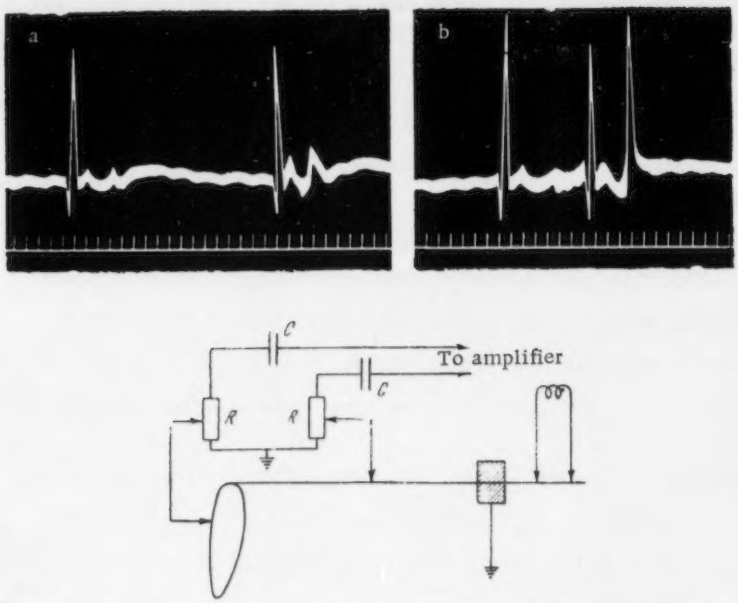

Fig. 3. Summation of excitation in the transmission of two nerve electropotentials from the nerve to the muscle in a zone of botulism paralysis; a) absence of transmission of two nerve activity currents from nerve to muscle within an interval of 36 milliseconds; b) appearance of transmission of excitation from nerve to muscle as a result of summation within an interval of 13 milliseconds between impulses. Time mark – 500 cycles per second.

In the last series of experiments on frogs poisoned with botulism toxin the purpose was to determine whether the phenomenon of summation in the transmission of only the second impulse through the myoneural junction when the nerve is presented with two stimuli is associated with alteration in the functional condition of the paralyzed motor nerve. In 19 experiments, as presented in Fig. 3, we recorded simultaneously the activity currents from the greater tibial nerve and the gastrocnemius muscle.

Experimental results indicated that in the development of botulism paralyses, the disturbance of transmission of excitation from nerve to muscle is observed only 4 – 6 days after priming, i.e. at a time when recording of significant alterations in the functional properties of motor nerve trunks can be made. According to our data, summation in the transmission of two nerve activity currents occurred with an interval between them of 12 to 54 milliseconds (Fig. 3, a, b). In experiments performed on the 2nd – 3rd day after priming, as in the 16 controls on normal animals, the phenomenon of summation was not observed inasmuch as the appearance of nervous activity current was always accompanied by an electrical response of the muscle.

Partial disturbance in conductivity of excitation through the myoneural lamina in paralytic botulism syndrome is apparently associated with pronounced alterations in functional properties of motor nerve conductors only.

On the strength of the electrophysiological data obtained, we can come to the conclusion that botulism toxin paralyzes the motor centers of the spinal cord first. Such a paralysis of the nerve center is accompanied apparently by a severe disturbance in its trophic effect on peripheral motor nerve conductors which results in gradual development of significant alterations in functional properties of nerve trunks and myoneural junctions.

SUMMARY

The effect of botulism toxin on the electric acitivity of the spinal cord was studied by the electrophysiological method. The author has demonstrated on frogs and cats that in "local" botulism the mono-polysynaptic and electrotonic reactions are absent in the anterior roots of the spinal cord. The functional condition of motor nerves was examined. It was revealed that with development of botulism poisoning, especially in late stages, a significant prolongation of the phases of relative and absolute refractivity and decrease of lability take place.

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CAPILLARY CIRCULATION IN EXPERIMENTAL PLAGUE

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Circulatory disturbance is one of the basic symptoms of the plague. However, the mechanism of circulatory insufficiency in this infection remains almost uninvestigated. Morphologic investigations in plague point to a considerable involvement of blood vessles, especially capillaries [1, 3-5]. Physiological verification of the physiological state of the various divisions of the vascular system in plague remains undone.

Taking into consideration the important role of capillary blood flow in the general circulatory system, we decided to investigate the character of the capillary blood circulation at various stages of experimental plague infection. Changes in the intensity of tissue circulation were evaluated by the alteration in the resorption of substances of low molecular weight from the site of infection. The latter was determined with the method of labeled atoms.

It is known that compounds of low molecular weight are removed from the site of injection mainly by the system of blood capillaries, whereas compounds of high molecular weight – principally by the system of lymphatic capillaries [2, 6, 7, 9]. Therefore the rate with which a radioactive isotope combined with a compound of low molecular weight is removed from tissue can serve, to a certain degree, as an index of the status of capillary blood circulation. Under these conditions the absorption time depends on the intensity of blood circulation, the number of functioning capillaries and the state of capillary permeability [8, 10].

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs. A hypodermic injection of 0.1 ml solution of $Na_2HP^{32}O_4$ was given with a tuberculin syringe into the previously hair–clipped area of the abdominal wall. The concentration of P^{32} was chosen so that the number of impulses at the beginning of the recording would be within the limits of 1,000 per minute. Impulses were recorded with apparatus B equipped with counter AC – 2, which is enclosed in a lead membrane with an aperture 12 mm in diameter. The counter was situated above the site of injection of the radioactive substance and 1 cm from it. The intensity of irradiation was recorded at one minute intervals throughout the experiment.

Half-absorption time or the time necessary for absorption of half of the quantity of isotope introduced was used as an index of the rate of resorption. The process of resorption was depicted graphically in a system of semilogarithmic coordinates: the abscissa axis – time in minutes, the ordinate axis – logarithm of the number of impulses. The half-absorption time was calculated from the graph. The data obtained was processed statistically.

The intensity of the resorption of the isotope introduced was determined 3-4 times on healthy guinea pigs. The animals were then infected by the subcutaneous injection of a virulent strain of B. pestis 773. The infected dose consisted of 1000 microbes which is equal to 100 Mld. This was followed by daily determinations of the status of capillary blood flow in the dynamics of the plague infection.

Simultaneously, an investigation of the pathologico-anatomical and bacteriological picture of the

infectious process was carried out by daily autopsies of 1-2 pigs following the determination of the rate of resorption of the isotope introduced. This clarified the character and degree of pathologico-anatomical changes and at the same time agar media were inoculated with subcutaneous tissue from the site of the infected regional lymph node, and from the spleen, liver, lungs and blood.

EXPERIMENTAL REULTS

In experimental plague infection retardation of the resorption rate of $Na_2HP^{32}O_4$ was observed. Table 1 shows that within 24 hours after infection the half-absorption time was prolonged in all guinea pigs receiving the isotope. If prior to the infection the average half-absorption time was 12.1 minutes, 24 hours after infection it was found to be 20.3 minutes. Statistically this difference is entirely reliable ($P < 0.001$).

TABLE 1

Study of the Rate of Resorption of $Na_2HP^{32}O_4$ Introduced Subcutaneously in the Process of Developing of Experimental Plague Infection

Guinea pig number	Half-removal time of isotope at various periods following infection							Number of days between infection and sacrifice or spontaneous death
	prior to infection	period following infection (in days)						
		1	2	3	4	5	6	
1	10	21	—	—	—	—	—	1 (s)
2	13	20	27	—	—	—	—	2 (s)
3	10,5	29,5	55	54	—	—	—	3 (s)
4	12,5	18	25	29	—	—	—	3 (s)
5	11,5	19	31	36	43	—	—	4 (s)
6	9,5	18,5	43	54	—	—	—	3
7	10,5	19	23	32	52	—	—	4 (s)
8	12	21,5	29	37	49	67	—	5
9	11,5	22,5	27	43	58	—	—	4
10	14,5	19	34	58	—	—	—	3
11	12	16,5	19	29	32	38	—	5
12	9,5	14,5	24	37	43	65	—	5
13	12	23	37	44	55	—	—	4
14	15	16	31	51	62	—	—	4
15	16	23	28	39	44	70	—	5
16	13,5	20,5	28	34	49	61	—	5 (s)
17	9,5	19	31	36	41	—	—	4
18	14,5	16	29	40	44	49	60	6 (s)
19	10,5	27	37	55	—	—	—	3
20	14	24	31	43	67	68	—	5
M	12,1	20,3	29,4	44	49,1	59,7	60,0	—

Prearranged designations: M — arithmetical mean values; y) sacrificed.

During this period of the infectious process pathologico-anatomical changes were not observed and a culture of B. pestis was isolated only from the site of injection (Table 2).

Two days after infection resorption time exceeded the initial by more than twice. Plague microbes were found at the site of injection and in the regional axillary lymph nodes. The pathologico-anatomical picture is limited by a slight edema of the subcutaneous tissue and by punctate hemorrhages in the regional nodes.

The first changes in the viscera in the form of enlargement of lymph nodes, and hyperemia of the liver and spleen were observed on the 3rd — 4th day. Four days after infection plague microbes were found in all organs examined. Isotope resorption time at this stage of the disease exceeded the initial by more than 4 times As the infectious process progressed, the resorption time progressively increased and on the 5th day of the disease reached the mean of 59.7 minutes, i.e. almost 5 times greater than the initial time.

Especially abrupt was the decrease of the absorption of isotope in terminal guinea pigs (half-removal isotope time – 65 – 70 minutes). Taking into consideration the fact that the rate of resorption of substances of low molecular weight reflects the intensity of tissue circulation, one may assume that in experimental plague infection there is an abrupt lowering of the intensity of peripheral blood circulation.

TABLE 2

Rate of Resorption of Isotope, Infectiousness of Organs and Pathologico-anatomical Changes in the Dynamics of Plague Infection

Animal number	Days between infection and sacrifice of animal	Half-removal time in minutes		Inoculation of B. pestis from organs						Localization of pathologico-anatomical changes
		prior to infection	on the day of autopsy	site of injection	lymph node	spleen	liver	lung	blood	
1	1	10	21	+	–	–	–	–	–	Not indicated
2	2	13	27	+	+	–	–	–	–	LN
3	3	10,5	54	+	+	+	+	+	+	LN, S
4	3	12,5	29	+	+	+	+	–	–	LN, L
5	4	11,5	43	+	+	+	+	+	–	LN, S, L
7	4	10,5	52	+	+	+	+	+	+	LN, S, L
16	5	13,5	61	+	+	+	+	+	+	LN, S, L, Ls
18	6	14,5	60	+	+	+	+	+	+	LN, S, L, Ls

Prearranged designations: – B. pestis culture isolated; – B. pestis culture not isolated; LN) lymph node; S) spleen; L) liver; LS) lung.

Comparison between the inteisity of absorption of isotopes and the dynamics of infectiousness of organs and their pathologico-anatomical changes at various stages in the course of experimental plague infection showed that disturbances of absorption and, therefore, of capillary blood circulation appear considerably earlier than the visible pathologico-anatomical changes and precede the distribution of plague microbes from the site of injection into the blood and viscera.

SUMMARY

Capillary circulation was examined in experimental plague of guinea pigs. The rate of removal of radioactive phosphorus beyond the limits of the site of injection served as an indicator of the intensity of capillary circulation in healthy and infected animals.

During plague the rate of resorption of radioactive phosphorus is considerably diminished, which points to the decreased intensity of capillary circulation. Disturbances of circulation occur before the appearance of any visible pathologico-anatomical changes and precede the entrance of plague bacilli into the internal organs and blood.

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APPLICATION OF THE SUPRAVITAL STAINING METHOD TO THE STUDY OF THE SORPTIVE PROPERTIES OF CERTAIN ORGANS IN EXPERIMENTAL TUBERCULOSIS

PART IV. SORPTIVE PROPERTIES OF KIDNEY AND SUPRARENAL GLAND TISSUES

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The objective and comparative evaluation, both quantitative and qualitative, of the degree of injury suffered by the cells of various tissues of animals during the course of tuberculosis is of great importance for the study of the pathogenesis of this disease. We have shown that such an evaluation may be achieved by means of the supravital staining method of D. N. Nasonov and V. Ia. Aleksandrov, in conjunction with the histological examination of the tissues. Our investigations of the sorptive properties of the cells of the cerebral cortex, skeletal muscles, spleen, and lungs of guinea pigs have revealed changes in the functional state of tissue elements, which are evident within a few hours of inoculation of the animals with tuberculosis, and have also shown how these changes develop as the disease progresses.

Our results showed the possibility of investigating the changes in sorptive properties of other tissues of experimental animals infected with tuberculosis.

The present paper describes the results of a study of the sorptive properties of the kidneys and adrenals of animals suffering from experimental tuberculosis, of different degrees of severity.

We have been unable to trace any literature references to any similar researches. The published material deals with the evaluation of specific and unspecific changes in these organs in various forms of tuberculosis (these papers are both of the clinical and of the experimental type [5, 6, 7, 10, 12, 19, 20, 21, 23, 25]). They give only a qualitative evaluation of the changes observed, and apply mostly to advanced stages of the disease [1, 8, 9, 11, 13, 15, 16, 22]. Physiological investigations were restricted to overall renal function, and more recently to adrenal function [2, 3, 4, 14, 17, 18, 24].

EXPERIMENTAL METHODS

We used 410 guinea pigs, and carried out 800 examinations (400 of the kidneys, and 400 of the suprarenal glands). The animals were divided into 3 groups, of which the first was inoculated subcutaneously (in the region of the right axilla) with a suspension of M. tuberculosis (bovine type, Strain 109), at a dosage level of 0.01 mg in 1 ml of physiological saline (the most severe degree of infection); the animals of the 2nd group were given subcutaneous injections of BCG culture, at a dosage level of 1 mg in 1 ml of physiological saline (the mildest degree of infection), while the animals of the 3rd group were inoculated with the virulent culture after having

*Deceased.

previously been immunized with BCG culture (two vaccinations; the disease was of a medium grade of severity in these animals).

The kidneys and adrenals were removed together with the capsules, and were placed on muslin moistened with Ringer's solution. The capsule was carefully stripped from the kidneys. The organs were then immersed for 10-15 minutes in Ringer's solution, in order to 'refresh' them. They were then immersed for 30 minutes in 0.01% neutral red solution at 20-22°; the kidneys were suspended in the dye solution. After staining, the organs were rinsed in Ringer's solution, stumps of blood vessels were removed, and the organs were suspended in a fixed volume of acidified alcohol (70% alcohol, acidified with 2% sulfuric acid; the volume was the same for each organ). The organs were removed after 24 hours, dried to constant weight, and weighed on a torsion balance. Optical absorption of the extracts was measured in a Pulfferich step-photometer, and was recorded per gram dry weight of the organ. The determinations were made at the same time intervals after inoculation as in our previous researches.

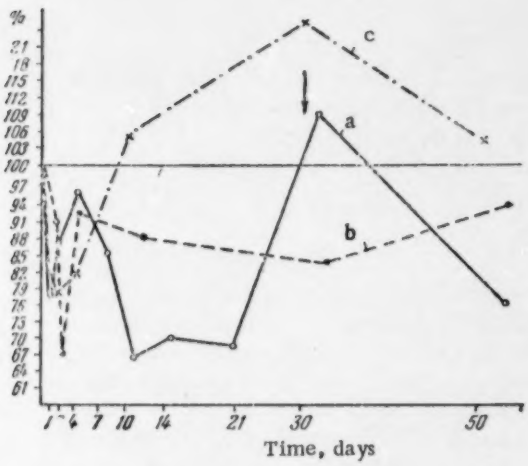

Fig. 1. Changes in sorption of neutral red by the kidneys of guinea pigs suffering from experimental tuberculosis. Abscissae: time in days after inoculation; ordinates: amount of neutral red bound by the organ, expressed as percentages, taking 100% as the amount bound by a kidney of a control animal. The arrow indicates the day on which solitary tubercles were first observed. a) After inoculation with a virulent culture of bovine type tuberculosis germs; b) after inoculation with an attenuated BCG culture; c) after inoculation with virulent culture, following previous immunization (two vaccinations).

Parallel with determinations of sorptive capacity we made histopathological examinations of the organs taken from guinea pigs which had been set aside for this purpose. We found (although not in every case) that solitary tubercles appeared in sections of the kidneys on the 30th day after inoculation with virulent cultures of M. tuberculosis. No pathomorphological changes could be seen in the kidneys before then, or in the adrenals at any time after inoculation.

EXPERIMENTAL RESULTS

It was necessary, in order to evaluate the sorptive properties of kidney and adrenal tissues of animals suffering from tuberculosis, to know how the dry weight of these organs varied during the course of the disease. Our experiments showed that significant variations in the dry weight of these organs were only encountered when the animals were inoculated with virulent culture. Increase in the dry weight of the kidneys was first noted on the 30th day after inoculation, and by the 50th day it was 50% higher than in the control animals.

We found (Figs. 1 and 2) that the supravital staining of the kidneys and adrenals showed considerable fluctuations during the course of the tuberculous process. The sorption curves for the kidneys (Fig. 1) and the adrenals (Fig. 2) of the animals of all 3 groups of experiments merit special attention, since they differ radically from those previously found by us for the brain, muscles, spleen, and lungs (Parts 1, 2, and 3 of this series). Whereas we then found an increase in the staining capacity of the brain and muscles, and particularly of the spleen and lungs, we now observed a marked fall in sorptive capacity of the kidneys and adrenals of similarly infected animals.

Sorption of neutral red by kidney tissue proceeded differently in each of our three experimental groups. In the group inoculated with BCG culture the sorption level remained below that of the control group over the whole period of observation. In the group infected with virulent culture the sorptive capacity fell to a very low level until the 21st day after inoculation, when it rose somewhat (to +9%) on the 30th day. It is of interest that the peak value coincided in time with the appearance of tubercles, and with a rise in the dry weight curve. In the third group, in which the guinea pigs were given virulent culture after having previously been immunized by two vaccinations with BCG culture, the sorption level rose above that of the control group on the 10th day

after inoculation; by the 30th day it exceeded this level by 25%, and was 16% higher than that of the group inoculated with virulent culture alone.

The first phase of lowering the sorptive capacity of the kidneys is identical with that found earlier for brain, muscle, spleen, and lung tissues. Sorptive capacity fell to a minimum of -35% on the 10th day after inoculation, in the first group of experiments. In the second and third groups minimum values were found after 48 hours and 24 hours, respectively. As is evident from Fig. 2, the sorption curves are of an undulating nature.

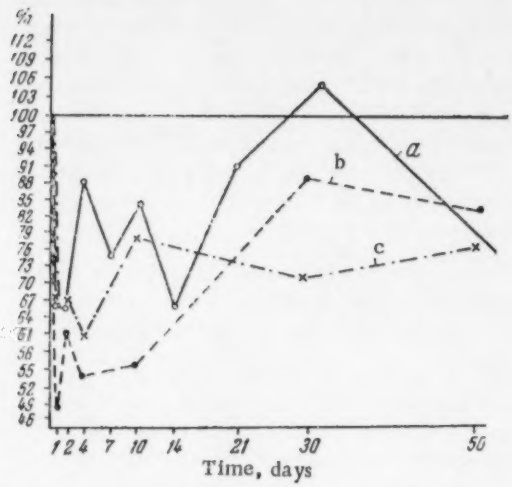

Fig. 2. Changes in sorption of neutral red by the adrenals of guinea pigs suffering from experimental tuberculosis. Abscissae: time in days after inoculation; ordinates: amount of neutral red bound by the gland, expressed as percentages, taking 100% as the amount bound by the glands of control animals. Designation of curves as for Fig. 1.

The differences in the nature of the reactions of the kidneys to infection are not restricted only to quantitative differences in the degree of lowering of sorptive capacity in the three groups. At no time after inoculation with BCG culture did we observe a sorptive capacity higher than that of the control group. The sorptive capacity of the kidneys of guinea pigs infected with virulent culture exceeded that of the controls on one occasion only (on the 30th day), by only 8.6%. It was at this time that the appearance of solitary tubercles was noted. The sorptive capacity of the kidneys of guinea pigs inoculated with virulent culture after having been immunized exceeded the control level on the 10th and the 30th days (unfortunately no determinations were made between these days), attaining a fairly high level on the 30th day (+ 25%). Binding of neutral red by adrenal tissues was much less than in the control series for all three groups (see Fig. 2). The lowest values were encountered in the group inoculated with BCG culture, being 52% of the control level 24 hours later. The sorption curves for all three groups resembled those found for the kidneys. Very slight increase in sorptive capacity of the adrenals was found only in the group given virulent culture, reaching +5% on the 30th day, after which it again fell below the control level. In the remaining two groups it was at all times lower than in the control series.

It may be concluded from our results that the adrenals possess extraordinarily high powers of adaptation to the changed conditions prevailing during the course of tuberculous infection; this did not apply to any other organ examined by us.

In concluding this paper, which is the last of the series of communications, we think it desirable to summarize our findings, the more so as they apply to a number of different organs of animals of the same species.

As is known, it has been shown by D. N. Nasonov and his co-workers that injuries inflicted on the living cells of a number of isolated organs by a wide variety of physical and chemical agents lead to enhancement of the affinity of protoplasm for basic and acidic stains. It has been shown that this unspecific reaction varies in intensity parallel with the gravity of the injury inflicted on the cell.

As far as we are aware, our researches are the first in which it has been attempted to investigate the reactivity of the tissues of whole organisms during the course of a pathological process; our results show that the supravital staining method can be applied to the quantitative evaluation of the damage suffered by tissue elements of different organs at different stages of development of the tuberculous process in experimental animals. Marked changes in the sorptive properties of the tissues were found to occur within the first few days after inoculation, before there were any anatomical signs of the disease, both in organs which were subsequently affected by the specific inflammatory process (lungs, spleen, kidney), and in those not so affected (brain, muscle,

adrenal glands). As observed in isolated organs, these changes vary in intensity, according to the gravity of the disease, which differs from group to group of animals, and exhibit fluctuations as the disease progresses. These fluctuations have the common feature of being of a phasic nature; the changes in reactivity seen in the first phase of lowered affinity for the dye reflect the process of adaptation of tissue elements to the complex of noxious factors (direct and reflex) which come into play during the progress of the morbid process. Paranecrotic changes then supervene, associated with rise in sorptive capacity, and, judging from our results, these changes may be reversible. Only in the advanced stages of the grave form of the disease, and in those organs which are the most susceptible to injury by the given pathogen, do substantial irreversible changes appear in the protoplasm.

We would emphasize that the supravital staining procedure of D. N. Nasonov and V. Ia. Aleksandrov has permitted of the establishment of the following: 1) the existence of changes in the functional state of the tissue elements of organs in which such changes cannot be perceived by any other procedure, or in which they only appear at an advanced stage of the disease (brain, muscles, adrenal glands); 2) the existence of changes in the functional state of all the organs examined following vaccination with BCG culture, although at no time subsequent to vaccination could any specific morphological changes be detected; 3) the existence of qualitatively and quantitatively dissimilar changes in the sorptive properties of different organs, side by side with features common to all of them; this permits of the drawing of conclusions regarding the dynamics of the morbid process, and of the order in which these organs are involved in the tuberculous process.

We conclude from our observations that at the stage at which extensive anatomical changes make their appearance in a given organ (changes in its weight and in the qualitative composition of its tissue elements), the study of sorptive properties becomes pointless, as the data obtained are not comparable with those of the controls or with the preceding state of the organ.

Although we are not inclined to consider that the results of our study of the physiological state of cells of animals suffering from experimentally induced tuberculosis are necessarily specific for this disease, the possibility cannot be excluded that they may have some pathognomonic significance for tuberculosis.

SUMMARY

Sorption of neutral red by the isolated kidneys and adrenals of guinea pigs has been determined, following inoculation of the animals with virulent or BCG cultures of bovine type M. tuberculosis, as well as after inoculation of immunized animals with virulent culture. In contrast to other organs, the sorptive capacity of these organs falls considerably after inoculation, although similar fluctuations are seen subsequently in the sorption – time curves as with other organs (brain, muscle, spleen, lungs) during their phase of heightened affinity for the stain. Maximum sorption of neutral red coincides in time with appearance of tubercles in the kidneys, spleen, and lungs. In the kidneys, inoculation of immunized guinea pigs with virulent culture is followed, after an immediate fall in sorptive capacity, by a sustained rise to levels higher than those found in the control series or in nonimmunized animals infected with virulent culture.

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EXPERIMENTAL STUDY OF ACUTE-PHASE REACTIONS

PART I. Cx-REACTIVE PROTEIN

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Serious attention has been paid of recent years to the so-called acute-phase reactions. These are related to the appearance in the blood of persons in the acute phase of various infectious and noninfectious diseases of a protein, which is not found in the blood of healthy persons. This protein has the property of reacting with the somatic pneumococcal polysaccharide, which is designated as C-polysaccharide, and for this reason it was called C-reactive protein.

The appearance and disappearance of C-reactive protein in the serum are closely connected with the exacerbation or extinction of inflammatory processes, and its content in the blood is an index of the activity of the morbid process.

The presence of C-reactive protein was first reported by W. Tillet and T. Francis [4] in pneumonia patients. The protein disappeared from the blood after the crisis had passed. The originally held view that C-protein is an antipneumococcal antibody was not confirmed. Shortly after, T. Francis [4] and T. Abernethy found this protein in the blood of patients suffering from acute rheumatism. C-reactive protein was first isolated from the blood serum of patients by C. MacLeod and O. Avery [3], and later by M. McCarty [2] from inflammatory exudates, and prepared in crystalline form.

Immunologically, this protein differs from other serum proteins. Immune serums obtained from rabbits which had been immunized against this protein contained the corresponding antibody, but they did not react with normal human serum. This finding permitted of the detection of C-reactive protein in the serum of patients.

G. Löfström's finding [1] that a protein analogous to C-reactive protein was present in the blood of rabbits suffering from acute inflammatory processes was of great importance for the study of acute-phase reactions. The rabbit protein did not react with pneumococcal C-polysaccharide, but it reacted with a related polysaccharide, which was prepared by rapid lysis of the bacteria, followed by removal of proteins. This polysaccharide was called Cx-polysaccharide, and it is thought to be a polymeric form of the somatic pneumococcal polysaccharide, termed C-polysaccharide (M. McCarty).

It was found that the serums of patients reacted with both of these polysaccharides, whereas those of infected rabbits reacted only with the Cx-polysaccharide. For this reason, the corresponding protein component of acute-phase rabbit serums was called Cx-reactive protein.

The above findings made it possible to study acute-phase reactions in animal experiments, and to attempt to use Cx-reactive protein antiserum clinically for the detection of C-reactive protein in the blood of patients.

The present research was undertaken with the following objects: 1) to ascertain the conditions and the

dynamics of appearance of Cx-protein in the blood of rabbits in response to various stimuli; 2) to attempt to obtain the corresponding antiserum; 3) to find whether the Cx-proteins formed under different conditions are immunologically identical.

EXPERIMENTAL METHODS

In our first series of experiments intracutaneous injections of a virulent culture of Type I hemolytic streptococcus were given to a group of 40 rabbits. We used an 8-hour meat broth culture, which was given undiluted in some cases (from 1 to 6 injections of 0.2 ml each), and in other cases at dilutions of 1:10 and 1:100. A marked inflammatory reaction was seen within 16-18 hours at the site of injection, where necrotic changes appeared subsequently. Some of the animals, which had been given undiluted culture, died within a few days, with symptoms of generalized sepsis. In the second series of experiments (17 rabbits) the animals received intracutaneous injections of live H. pertussis organisms, the inoculum of 16 billion microbial bodies being administered in a course of 20 injections; other animals of this series were given toxic whooping cough vaccine, at the same dosage levels. Pronounced hemorrhagic inflammatory reactions, followed by necrosis, were seen at the injection sites. In some of the experiments the animals were given diphtheria toxin, in doses of 1.5 Mld (guinea pig), in 5 injections. These injections too, were followed by a marked localized inflammatory reaction and by skin necrosis. The Schwartzman phenomenon was produced in 5 rabbits, by giving them 5 intracutaneous injections of E. coli filtrate (each 0.2 ml), followed after 24 hours by an intravenous injection of the same filtrate, at a dosage level of 2 ml per kg body weight. Finally, 2 rabbits were given intravenous injections of a pyrogenic preparation (Pyrogenal), in a dose of 5μg per kg body weight, which caused a transient febrile reaction.

TABLE 1

Times of Appearance and Disappearance of Cx-Protein in the Blood of Rabbits Inoculated Intracutaneously with Streptococcus Culture

Time of taking blood sample (hours)	Rabbits inoculated with culture in 1:10 dilution (5 injections)					Rabbits inoculated with culture in 1:100 dilution (5 injections)				
	1	2	3	4	5	6	7	8	9	10
4	++	+	.	.	.	.	.	±	±	+
8	++	+	.	.	.	.	.	+	±	.
18	.	++	++++	+++	++++	++	++	.	.	+
24	.	+++	+++(+)	++	+++	++	++	+++	++	+++
48	.	+	++	++	++	++	+++	++	+++	+
72	.	—	++	+(+)	+	++++	.	++	++	+
4 day	.	—	+	+(+)	+	++	.	+	+(+)	+
5—6 days	.	—	—	—	±	—	.	.	—	—

Explanation of symbols in this and in subsequent Tables: +++ and ++++ designate strongly positive reactions; ++ positive reactions; + weakly positive reactions; . tests not performed.

Repeated blood examinations were made, at times from 4 hours after beginning the experiments up to 6 days. We applied the complement-fixation test (prolonged fixation at +2°). The antigen was C-polysaccharide, prepared from a pneumococcal culture either by hydrolysis with HCl (by Lancefield's method), or by McCarty's method, with the difference that lysis of the pneumococci was achieved by adding ox bile instead of sodium desoxycholate. The Lancefield antigen was taken in 1:20 dilution; the McCarty antigen was dissolved to give a concentration of 1 mg/ml, and this solution was then diluted 1:50. The blood serums were used in dilutions of from 1:10 to 1:80.

The results of the tests were designated as positive (++) when total inhibition of hemolysis was found with not less than $1\frac{1}{2}$ doses of complement, and as strongly positive (++++) when a double dose of complement was taken. Blood samples taken from the same rabbits before the experiment served as controls.

EXPERIMENTAL RESULTS

Cx-reactive protein was found in 36 of 40 rabbits in which localized inflammatory reaction had been produced by injection of streptococcal suspensions. Of 87 blood serums examined, negative results were obtained in 12, and definite or strongly positive reactions (++ or ++++) in 61 cases; weakly positive results were obtained in 14 cases.

TABLE 2

Specificity of Cx-Reactive Protein Antiserums

Antigens ("acute-phase" rabbit serums)	Experiments using non-neutralized immune serum	Experiments with neutralized immune serum	
		Normal rabbit serum	"Acute-phase" rabbit serum
№ 31	++	++	—
№ 33	++	++	—
№ 37	++	++	—
№ 6	++++	++++	—
№ 7	++++	++++	—

Cx-reactive protein was found in the blood of all 17 of the animals of the second series of experiments. Negative reactions were found with 29 of 86 blood samples, definite or strongly positive reactions with 36 samples, and weakly positive reactions with 21 samples. The larger number of negative reactions found in the second series may be ascribed to the tests being performed at both earlier (4 hours) and later (5-6 days) times than in the first series.

TABLE 3

Reactions of Native and Neutralized Serums with Heterologous and Homologous Cx-Proteins

Antigens ("acute-phase" serums)	Immune serums from rats immunized against "acute-phase" rabbit serums											
	No. 2 37 and 34				No. 31 and 33				No. 6 and 7			
	non-neutralized	neutralized serums			non-neutralized	neutralized serums			non-neutralized	neutralized serums		
		№ 37 and 34	№ 31 and 33	No. 6 and 7		№ 37 and 34	№ 31 and 33	No. 6 and 7		№ 37 and 34	№ 31 and 33	No. 6 and 7
From rabbits inoculated with streptococcal culture — serums:												
№ 2	++	—	—	—		—	—	—	++	—	—	—
№ 37		—	—	—	++++	—	—	—	+++	—	—	—
№ 34	++	—	—	—	++++	—	—	—		—	—	—
From rabbits given H. pertussis culture — serums:												
№ 31	++	—	—	—	+++	—	—	—	++	—	—	—
№ 33	++	—	—	—	+++	—	—	—	++	—	—	—
From rabbits showing the Schwartzman phenomenon — serums:												
№ 6	++	—	—	—	++++	—	—	—	+++	—	—	—
№ 7	++	—	—	—	+++	—	—	—	+++	—	—	—

The times of appearance and accumulation in the blood of Cx-reactive protein corresponded with the evolution of the inflammatory and febrile reactions. Thus Cx-reaction protein appeared soonest in the serum of rabbits which had been given Pyrogenal. Rise in temperature was seen after 1 hour, and a definite positive (++) reaction for Cx-protein was obtained after 4 hours, remaining at this level for a day. In animals suffering from localized inflammatory-necrotic processes due to intracutaneous introduction of H. pertussis culture or vaccine, or of diphtheria toxin, the highest contents of Cx-reaction protein were found after 24-72 hours, i.e at the times taken for development of the skin lesions; most of the rabbits giving the Schwartzman phenomenon showed maximum concentrations of Cx-reactive protein within 24-48 hours. The times of appearance of Cx-protein in the blood of rabbits inoculated with streptococcus culture, and of its subsequent disappearance, are shown by the data of Table 1.

For the preparation of serum immune with respect to Cx-reactive protein we immunized rats with rabbit serum in which this protein was present. Four groups, each of 10-25 rats, were immunized with serum obtained from rabbits which had been inoculated intracutaneously with streptococcal culture. The animals of another 4 groups were immunized respectively with serum taken from rabbits which had been inoculated intracutaneously with live H. pertussis culture, diphtheria toxin, and Pyrogenal, and in which the Schwartzman phenomenon had been produced.

Immunization consisted of 6 weekly courses of 4 daily injections of antigen, with a 3-day interval between the courses. Intraperitoneal injections of antigen were given, in doses of 0.01 ml per injection during the first week, and of 0.015 ml per injection in subsequent weeks. Blood samples were taken 10 days after completing immunization; they were tested for complement fixation with the "acute-phase" serums, and also with normal rabbit serum. In most cases we were thus able to obtain serums which gave a sufficiently definite reaction with "acute-phase" serum, while giving only a low titer with respect to normal rabbit serum.

Apart from this, specificity of the results was ensured by preliminary neutralization of the immune sera with normal rabbit serum. Examples of this are given in Table 2.

We performed 2 series of experiments with the object of ascertaining whether or not the Cx-proteins appearing in the blood of rabbits suffering from inflammatory conditions due to the action of various agents are immunologically identical. In the first series we tested various immune serums against "homologous" and "heterologous" Cx-proteins. In the second series we saturated immune serums with various "heterologous" Cx-proteins, and then tested the neutralized serums. Examples of these experiments are given in Table 3.

We found that each of the rat immune serums gave an equivalent reaction with all the rabbit "acute-phase" serums, irrespective of the nature of the agent responsible for the inflammatory reaction in both groups of rabbits. The corresponding antibodies were removed from the immune serums after saturation with any of the "acute-phase" serums. It may hence be concluded that the Cx-proteins appearing in the blood of rabbits suffering from inflammatory conditions caused by various agents are immunologically identical.

SUMMARY

The ability of acute-phase rabbit serums to react with somatic pneumococcal polysaccharide has been confirmed. The complement-fixation test is applicable to the detection of this reaction.

Immune serums were obtained from rats, which reacted specifically with rabbit acute-phase serums, but only weakly with normal rabbit serum. The Cx-reactive protein appearing in the blood of rabbits in the acute phase of inflammatory reactions to inoculation with various agents (pneumococcus and H. pertussis cultures, diphtheria toxin, E. coli culture filtrates, pyrogens) was immunologically identical, irrespective of the nature of the agent used.

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BIOCHEMISTRY AND BIOPHYSICS

THE EFFECT OF A PULSED SUPER-HIGH FREQUENCY (SHF) ELECTROMAGNETIC FIELD ON THE HIGHER NERVOUS ACTIVITY OF DOGS

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The effect of an alternating electromagnetic field on the higher nervous activity is a question that has repeatedly attracted the attention of research workers. The study of conditioned reflexes in animals exposed to a super-high frequency field has been carried out, for instance, by N. S. Kharchenko [6], D. Ia. Glezer [1], N. N. Livshits [3] and T. N. Promtova [5].

However the function of the higher divisions of the central nervous system has evidently not been studied in super-high frequency field conditions (centimeter and millimeter wave-bands); in experiments carried out on a single dog by V. A. Alekseev, he was unable to obtain any conclusive results.

Meanwhile the problem of the effect of an SHF field on the cerebral cortex is one of great interest, since microwaves are being used more and more widely in technology and medicine. In addition, persons working with microwave generators are particularly prone to complain of quickly becoming tired and sleepy.

The aim of the present investigation was to determine the changes in the higher nervous activity of dogs during single and prolonged exposure to an SHF field of varying intensity.

EXPERIMENTAL METHODS

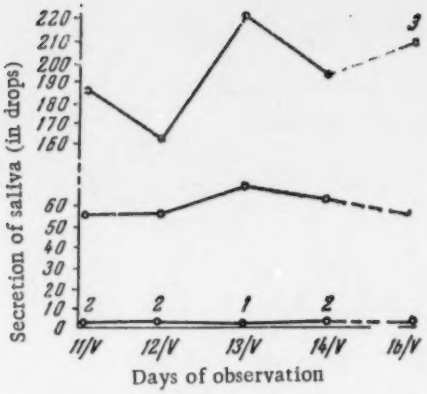
Experiments were carried out on 7 dogs by the classical food-saliva method of I. P. Pavlov. The secretion of saliva in 5 dogs was recorded by means of S. V. Zhevlakov's funnel, improved by us in conjunction with Iu. P. D'iakov. By means of a relay, this funnel enabled the number of drops of saliva to be recorded on paper. In the two remaining dogs the secretion of saliva was recorded on a Ganike-Kupalov scale.

The dogs Jim, Ryzhik and Dick were used first in the experiment; Jack, Trezor, Bel'chik and Orlik had been used earlier to study the interaction of extero- and interoceptive conditioned reflexes. In each dog a stereotype was established and consolidated (see Tables 1 and 2), and the type of its nervous activity determined.

The experiments began with accustoming the animals to the irradiation apparatus. The need for this was due to the fact that the noise of the SHF field generator was quite loud and could have an effect on the conditioned reflexes.

After extinction of the orientational reactions we set about irradiating the animals. For this purpose the dog was placed with the right or left side of its body in front of the SHF field emitter. The distance between the emitter and the animal was varied. The time of action did not exceed 2 hours.

On terminating the irradiation the dog was let out and allowed to walk about for 10-15 minutes, and only after this was it placed in a sound-proof chamber where, in the usual routine, the conditioned reflexes were investigated, always at the same time. From 4-12 days later the irradiation procedure was repeated. In this way the effect of various numbers of exposures was ascertained.



Changes in the secretion of saliva in the dog Trezor in response to a negative (1) and a positive (2) stimulus. Total volume of saliva in 40 seconds with food reinforcement of each signal (3).

In 2 dogs, in addition, the effects of a large number of exposures were studied. For this purpose the animals were exposed daily for over 50 days to the action of the SHF field. Measurements of the intensity of the SHF field at the points where the dogs were placed were made by physicist Iu. P. D'iakov.

EXPERIMENTAL RESULTS

Single exposures. The first experiments already showed that a weak SHF field, causing no signs of heating of the animals (raised temperature, increased rate of respiration, etc.), leads to obvious changes in higher nervous activity. The character of these changes depends on the intensity of the SHF field, the duration of irradiation and the type of nervous system possessed by the animal.

For instance, immediately after irradiation for one hour, and particularly for 2 hours, at a field intensity of about 0.005 w/cm^2, an increase in the secretion of saliva was often observed to positive conditioned stimuli, with relative stability of differentiation. The latent period of the conditioned reflexes was shortened in the majority of cases. As an example may be cited the results of one of the experiments on the dog Trezor (strong, balanced, mobile type of nervous activity). In the figure are shown curves of total secretion of saliva in response to positive (curve 2) and negative (curve 1) stimuli. Curve 3 shows the total volume of saliva in response to food reinforcement of each signal (for 40 seconds). Table 1 gives a detailed picture of the same experiment, showing the results for each stimulus separately.

It can be seen from the results given that on the day of irradiation (May 13) the total volume of saliva in response to positive conditioned signals was increased by 24% (from 54 to 67 drops); on the next day the secretion of saliva was also somewhat raised (61 drops), and on the 4th day it was almost back to its normal level. This increase in the effect took place in response to the action of exteroceptive conditioned stimuli; an interoceptive positive signal [inflation of a rubber balloon in the rectum at the rate of once per second (balloon +)] caused the same volume of saliva both before and after irradiation – 11 drops. Differentiation to a metronome (60 beats per minute; M_{60}) and to the balloon[inflation of the same balloon at a rate of once in 5 seconds (balloon –)] was not disturbed. Unconditioned reflexes were increased immediately after irradiation by 37% in comparison with the basic level.

In Jack, Orlik and especially Bel'chik – dogs with an unbalanced type of nervous activity – phasic phenomena were observed in addition. These were expressed as an increase in the secretion of saliva, mainly on account of weak stimuli, which took place immediately after irradiation for 1-2 hours in a weak SHF field (intensity about 0.005 w/cm^2), and for this reason the volume of saliva in response to the positive signals was evened out. Differentiation either was improved if it was incomplete, or was inhibited if it was absolute (experiment by Z. P. Sukhova).

In the dog Dick (strong, balanced, mobile type) and Jim (weak type) under the same conditions of irradiation most commonly an opposite picture was observed: suppression of salivatory reactions to positive signals and inhibition of differentiation.

It must be emphasized that the effects of irradiation with an SHF field of low intensity depend largely on the initial functional condition of the cerebral cortex. In cases where the excitation of the cortex was lowered, obvious stimulating effects appeared. Where, on the other hand, the cortical activity was high, depression of the reactions to the positive signals and inhibition of differentiation took place. As an example we may quote the result of Z. P. Svetlova's experiment on the dog Ryzhik (weak type). In this dog, after prolonged working with the same stereotype of stimuli, drowsiness appeared when it was placed in the chamber, and the animal reacted hardly at all to conditioned and unconditioned signals.

TABLE 1

Results of Experiments to Investigate the Changes in the Higher Nervous Activity of the Dog "Trezor" When Irradiated in an SHF Field

Stereotype	Initial level				Irradiation for 1 hour		After irradiation			
	11/V 1955		12/V 1955		13/V 1955		14/V 1955		16/V 1955	
	latent period (in seconds)	conditioned reflex secretion of saliva (in drops during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops during 20 seconds)
M_{120}	3	12	3	10	3	15	4	11	5	11
Balloon	4	11	3	11	4	11	3	10	3	11
Bell	2	11	2	11	2	13	3	14	8	10
Light . . .	3	6	5	5	4	8	5	8	4	8
M_{60}	6	2	12	1	13	1	5	1	14	2
Balloon	—	0	16	1	—	0	15	1	—	0
Light	8	5	4	5	8	7	3	7	4	6
Bell	5	9	3	12	3	13	4	11	6	9
Total (in response to positive signals)	—	54	—	54	—	67	—	61	—	55

TABLE 2

Results of Experiments to Investigate the Changes in the Higher Nervous Activity of the Dog "Ryzhik" When Irradiated in an SHF Field

Stereotype	Initial level				Irradiation for 1 hour		After irradiation	
	7/VI 1957 r.		11/VI 1957 r.		12/VI 1957 r.		13/VI 1957 r.	
	latent period (in seconds)	conditioned reflex secretion of saliva (in drops) during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops) during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops) during 20 seconds	latent period (in seconds)	conditioned reflex secretion of saliva (in drops) during 20 seconds
Bell	5	2	—	0	3	12	12	6
M_{120}	—	0	—	0	6	5	6	2
M_{60}	—	0	—	0	—	0	—	0
Light	—	0	—	0	—	0	—	0
Bell	—	0	13	1	—	0	—	0

It is shown in Table 2 that on a background of sharp inhibition, irradiation with a weak SHF field completely restored the reactions to the two first stimuli. After the application of differentiation to one stimulus, disinhibition did not take place.

Prolonged irradiation. During prolonged irradiation of dogs with a weak SHF field (intensity about 0.005 w/cm^2) changes arise in their higher nervous activity similar to those observed during single exposures. However under these new conditions fresh details appeared. In the dog Jack (strong, balanced, mobile type), for instance,

TABLE 3

Conditioned Reflex Secretion of Saliva in the Dog "Jack" (in drop)

Stereotype	Initial level	Exposure for 2 hours a day, 36 hours altogether																						After effects							Additional exposures, 70 hours altogether			
	2/VI	3/VI	4/VI	6/VI	7/VI	8/VI	9/VI	10/VI	11/VI	13/VI	14/VI	15/VI	16/VI	17/VI	18/VI	20/VI	21/VI	22/VI	23/VI	24/VI	25/VI	27/VI	28/VI	29/VI	30/VI	2/VII	5/VII	16/VII	16/VIII	17/VIII	23/VIII
M_{120} . .	8	8	12	12	15	14	17	16	14	15	11	14	15	14	15	15	13	14	8	12	12	14	11	9	13	8	—	13	12	6	8
Light . .	5	5	8	10	9	13	14	12	13	13	12	10	10	15	12	12	11	8	9	9	11	10	10	9	8	9	—	6	6	8	8
Balloon+	8	8	9	9	14	13	14	16	14	14	11	12	14	14	14	13	10	10	8	12	8	11	11	10	9	8	—	8	8	2	2
Balloon	0	1	1	0	2	2	1	1	0	0	2	2	0	2	1	1	1	1	1	1	1	2	0	0	1	2	—	2	0	0	0
M_{60} . . .	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	—	0	0	6	6
M_{120} . . .	8	5	6	8	9	8	7	11	3	7	0	6	4	7	7	7	6	4	4	4	4	5	4	3	5	4	—	4	5	6	6
Light . .	3	6	7	7	12	11	6	8	10	10	7	7	7	11	11	10	9	9	8	7	7	8	7	7	8	7	—	4	5	6	8
Bell	10	10	11	10	13	14	15	12	14	15	13	11	14	12	12	13	13	10	13	12	13	13	13	12	10	12	—	10	8	9	9

in the course of the first 4-5 days, as irradiation was repeated (for 2 hours a day) a growth was observed in the conditioned reflex secretion of saliva from day to day (cumulation). Next, for several days the positive conditioned reflexes remained at a high level. Later on, as the exposures continued the magnitudes of the positive conditioned reflexes gradually fell to their original level, especially after an additional 35 day period of irradiation. Differentiation (M_{60} and balloon −) was disinhibited either not at all or only slightly.

From a comparison of the action of the conditioned stimuli separately it may be seen that, in the first place the secretion of saliva grew mainly on account of weak stimuli (Table 3). At the 4th-5th exposure, for instance, e.g. at the period when the effect of the irradiation has reached its peak, the secretion of saliva in response to the light stimulus was almost 3 times as great as that before irradiation; the secretion in response to M_{120} and to the balloon + was almost twice as great, and in response to the strongest stimulus − the bell − the increase in secretion of saliva was insignificant. As a result there was some approximation of the effects to weak and strong stimuli (equalizing phase). On some days paradoxical phenomena were observed. Such phasic effects were also observed on the last days of irradiation in the first series of experiments; however at the end of an additional 35 day period of irradiation there were no abnormalities in their strength relationships. In the second place, the secretion of saliva in response to an interoceptive stimulus (balloon +) did not increase on the 1st day, as in response to exteroceptive signals, but usually only on the 3rd and even on the 6th day. In the third place, in response to a positive stimulus M_{120}, following differentiation, the increase in the volume of saliva in the course of irradiation was far less than that to the same M_{120} when occupying a leading place in the stereotype. Starting with the 5th irradiation, the opposite picture could even be observed − depression of saliva secretion to this stimulus. This may clearly be seen from a comparison of the figures shown in Table 3 (compare M_{120} occupying first place in the stereotype).

Further, if some degree of disinhibition may be present, it was only observed in response to the first negative stimulus of a pair. The second differential signal (M_{60}) resulted in a zero reaction almost every day. Finally a shortening of the latent period of the conditioned reflexes could be observed, but only at the beginning of a large number of exposures.

After analyzing the material obtained we came to the conclusion that it was not possible to speak of

full restoration of conditioned reflex activity during continuing exposure to irradiation; only partial adaptation of the cerebral cortex to the action of the SHF field can be suggested.

In another dog, Jim, with a weak type of nervous activity, the changes in the conditioned reflexes were fluctuating in character: in the first 2 days of irradiation the volume of saliva secreted in response to a conditioned signal gradually fell, then grew for a few days, again fell and so on. Differentiation, which in this dog was incomplete, even improved during irradiation. However, as in the preceding dog, phasic changes were observed in this case also, with a gradual evening out of the conditioned reflex activity as the irradiation continued.

It must be pointed out that signs of adaptation were observed in the behavior of animals exposed to many repeated exposures to irradiation. These were shown by a certain restlessness of the dogs on the first days of irradiation only, after which, in spite of continuation of the exposures, this disappeared.

Thus a weak SHF field (intensity about 0.005 w/cm^2) when applied on single occasions or frequently, exerts mainly a stimulating effect on the conditioned reflex activity of animals. Of fundamental importance here are the number of exposures to irradiation, the original functional state of the animal and the type of its nervous activity.

Single intensive irradiation. An intensive SHF field (intensity about 0.1 w/cm^2), in contrast to a weak field, caused pronounced signs of heating of the animal (raised temperature, shortness of breath, salivation and so on). Under these circumstances the positive conditioned reflexes, especially in a dog of weak type, almost always were depressed, and differentiation was disinhibited. The latent period of the conditioned reflexes was usually lengthened. Phasic phenomena were seen even more commonly than with exposure to weak fields. Normalization of the conditioned reflex activity after single exposures of 2 hours to an intensive SHF field (intensity about 0.1 w/cm^2) appeared on the 4th-5th day. The unconditioned reflexes, on the other hand, were hardly changed or else increased.

The relationships described were shown quite clearly during irradiation both of the side from which the flow of saliva was drawn and of the opposite side.

Thus the direction of the changes during intensive irradiation was in the majority of cases opposite to that observed after exposure to weak fields.

The dependence of the effect of irradiation on the initial functional state of the animal was clearly evident during exposure to an intensive field also. In the same dog, Ryzhik, for instance, which as we have pointed out fell asleep in the chamber, the conditioned reflex activity was restored completely to normal by intensive irradiation: the positive conditioned reflexes were disinhibited and the animal began to take its food greedily.

Experiments with single exposures of dogs to irradiation show that a weak SHF field usually intensifies the excitatory process. Active inhibition is evidently more stable under these conditions.

Experiments with large numbers of exposures to irradiation indicate the possibility of adaptation of the cortex to the SHF field. However complete adaptation does not evidently take place to this stimulus, as shown by the phasic phenomena and the intensification of successive inhibition (a fall in secretion of saliva in response to M_{120} when applied after differentiation) during frequent exposures to irradiation. At the same time cumulation of the changes caused by irradiation obviously takes place.

An intensive SHF field, conversely, weakens both the excitatory and inhibitory processes, since it diminishes the magnitude of the conditioned reflex and disinhibits differentiation. These changes are most probably connected with the heating of the animal. A local thermal action of the SHF field on the salivary gland cannot, of course, be excluded; however this obviously has no essential significance, since if the area of the gland alone is irradiated the conditioned reflexes are unchanged.

It is more difficult to account for the action of the weak SHF field, in which neither the rectal not the subcutaneous temperatures are changed. Here either a specific thermal action (heating of only certain tissue structures) or a purely specific action of the electromagnetic waves may be postulated. In one way or another the changes in the higher nervous activity may follow along 3 lines: 1) on account of the direct penetration of electromagnetic waves into the brain; 2) reflexively, on account of stimulation of the receptor apparatus; 3) by a humoral-chemical route. This mechanism in respect of the SHF field has been suggested previously [2].

SUMMARY

The process of excitation is, as a rule, intensified in dogs on the background of relative stability on active inhibition in dogs submitted to a weak (pm about 0.005 wt per cm^2) SHF alternating power field for 2 hours (about 0.005 wt per cm^2).

Repeated numerous actions of such a field bring about development of phasic conditions. The phenomena of cumulation and partial adaptation to SHF field are observed. On the contrary, in high intensity of the action causing a thermal effect with SHF alternating power field (equal to about 0.1 wt/cm^2) a weakening of conditioned reflex reactions are observed with a disinhibition of differentiations.

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CHANGES IN THE DISTRIBUTION AND ELIMINATION FROM ANIMALS OF THE RADIOACTIVE HYDROCHLORIDE OF THE DIETHYLAMINOETHYL THIOESTER OF DIPHENYLOXYTHIOACETIC ACID CONTAINING LABELED SULFUR

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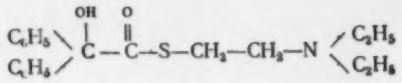
Considerable success has been achieved recently in the creation of new therapeutic preparations possessing a marked cholinolytic action. The most detailed studies have been made of spasmolytin – the diphenylacetic ester of diethylaminoethanol [3, 4, 5], the diphenylacetic ester of diethylaminoethyl-thioethanol – tiphen [1], and pentaphen – the diethylaminoethyl ester of diphenylcyclopentanecarbonic acid [2, 8].

The recently synthesized hydrochloride of the diethylaminoethyl thioester of diphenyloxythioacetic acid (thioester) is one of the members of this group of compounds:



Our aim was to study fate of this compound in animals, the character of its distribution in the body and tissues, and the route and rate of its elimination from the body.

EXPERIMENTAL METHOD AND RESULTS

For this purpose thioester containing radioactive sulfur S^{35} was synthesized.*

The preparation obtained is a white crystalline powder melting at 143-146°C and readily soluble in water and alcohol. The investigations were carried out in 4 species of animals: white rats, rabbits, cats and dogs, which were injected intramuscularly with a 1% aqueous solution of thioester–S^{35} in a dose of 5 mg/kg. The animals were killed 5, 30 and 60 minutes and 4 and 24 hours after injection of the compound, and organ tissues, plasma, urine and feces were taken for examination. The organs were thoroughly chopped up and ground in a mortar, and the homogeneous mass thus obtained was transferred to a glass plate and dried at a temperature of 65-70°C for 24 hours. The loss of weight was taken into consideration in the subsequent calculations. The dried tissues were ground to the consistency of a fine powder and sifted through a Kapronovoi sieve. Samples of 100 mg of the powder were transferred to targets to which were added 5-6 drops of alcohol and 0.5 ml of a 20% solution of KCl, which was essential in order to create a sufficiently large, dense residue on the surface of the target for the count to be made in the "thick layer." Next the targets were dried and their radioactivity measured. At the same time as the measurements were being made on the tissue sample, the radioactivity of

*The synthesis was carried out by Z. I. Bobysheva, to whom we express our gratitude.

a standard thioester solution was determined under the same conditions, thereby enabling the thioester concentration in the tissues examined to be calculated. The concentration was expressed in μg of thioester per 1 g of fresh tissue.

Content of S^{35} (Calculated as Thioester) in the Organs and Tissues of Rabbits and Cats at Various Intervals of Time after the Intramuscular Injection of the Preparation (in μg per 1 g of fresh tissue)

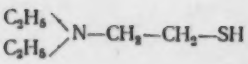
Organs and tissues	Cats	Rabbits	Cats	Rabbits	Cats	Rabbits	Cats	Rabbits	Cats
	5 minutes		30 min	60 minutes		4 hours		24 hours	
Cerebral hemispheres (grey matter)	1,61	2,80	9,70	4,60	4,71	2,64	1,50	0,81	0,50
The same (white matter)	0,57	1,07	6,41	2,43	2,88	1,88	0,89	0,73	0,46
Cerebellum	1,06	1,63	8,59	2,94	6,50	2,16	1,26	0,76	Traces
Corpora quadragemina	0,95	1,32	6,89	3,62	3,62	2,23	0,89	0,78	0,49
Optic thalamus	1,0b	1,23	6,17	3,09	0,46	2,21	0,93	0,76	0,41
Medulla oblongata	0,73	1,09	5,62	2,48	3,12	2,03	0,87	0,67	Traces
Spinal cord	0,47	0,71	2,35	1,69	1,79	1,40	0,37	0,45	»
Skin	—	0,42	2,49	—	2,23	—	2,56	—	»
Muscles	0,53	0,32	1,47	1,48	1,57	2,14	1,73	1,29	0,45
Liver	2,70	1,00	3,06	23,20	5,30	51,40	3,29	14,90	0,50
Heart	1,54	3,82	4,82	7,33	4,27	5,01	5,10	1,49	1,46
Kidneys	4,17	3,63	10,07	13,40	9,60	14,80	7,70	4,07	1,20
Lungs	16,82	22,40	32,30	21,30	21,20	18,80	11,40	2,46	0,90
Blood	2,33	2,74	3,12	6,04	3,41	7,77	1,77	1,29	0,46
Plasma	2,45	4,07	5,66	7,57	5,00	9,27	2,41	1,64	0,90
Urine	0,16	0,43	29,07	23,40	40,42	48,00	124,6	62,10	31,40

*Blood, plasma and urine in μg per 1 ml.

The character of the distribution of sulfur was found to be of the same type in all the animal species investigated. In content of S^{35} found in them, the lungs occupied first place, whereas the radioactive sulfur content of the central nervous system was comparatively small. This difference was particularly pronounced at short intervals of time after injection of the drug (see Table). A clearly marked relationship was observed between the quantity of S^{35} found and the time after injection of the preparation. Thus in the majority of organs the S^{35} content rose in the first 30 minutes and then began to fall progressively. The quantity of S^{35} in the urine rose considerably over a period of 4 hours. At the end of the first 24 hours the content of radioactive sulfur in the majority of organs became very low. Such a rapid elimination of radioactive sulfur from the organs led us to put forward the hypothesis that thioester does not take part in the formation of any stable compound with the biochemical tissue substrates, and rapidly leaves the body.

The question naturally arises, what is the nature of the radioactive sulfur found in the tissues after injection of thioester. Bernheim [6], Kisch and his co-workers [9] and Pulver [10] point out that the first step in the conversion of drugs with the structure of complex esters is their decomposition at the ester bond. Since thioester belongs to this particular class of compound, it might be suggested that it will be broken down in the body into its component alcohol and acid. If so, then it is possible that the radioactive sulfur found in the organs and tissues belongs not to the intact molecule of the preparation but to the product of its hydrolysis.

The most likely product of the hydrolysis of thioester to contain sulfur is diethylaminoethanethiol. For the purpose of establishing to what compound the radioactive sulfur detected in our experiments belongs, a series of investigations was carried out in which rabbits were injected with the product of hydrolysis of thioester labeled with sulfur – diethylaminoethanethiol (thioalcohol):



Diethylaminoethanethiol

Thioalcohol was injected in an equimolecular dose, and determination of the radioactivity was carried out at the same intervals of time as after injection of thioester.

As a result it was established that the character of distribution of sulfur differed after injection of thioester from that after injection of its product of hydrolysis – thioalcohol. This difference was particularly noted at early stages after injection (Fig. 1). The maximum quantity of S^{35} after injection of thioalcohol was found in the kidneys, whereas after injection of thioester of greater quantity was found in the lungs. In the divisions of the central nervous system the absolute quantity of sulfur after injection of thioalcohol was significantly greater than after injection of thioester. The findings obtained in this series lead us to consider that at short intervals after injection the S^{35} content reflects the pattern of distribution of the intact thioester molecule among the organs and tissues of the animal and that the preparation is present in the body mainly in an unsplit form.

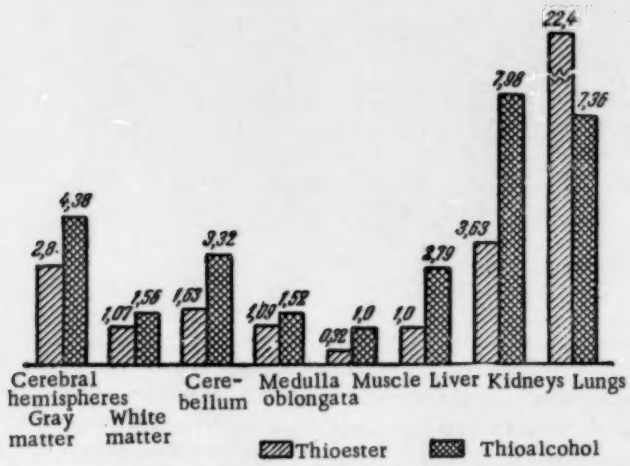


Fig. 1. Distribution of S^{35} in the organs of rabbits 5 minutes after the intramuscular injection of thioester and thioalcohol.

In the light of these findings it was interesting to study the ability of the tissues to carry out enzymic decomposition of thioester in order to ascertain whether it is possible in general for the enzymic hydrolysis of this compound to take place in the body.

For this purpose a series of experiments was set up in vitro, using the chemical method of determination of complex ethers suggested by Hestrin [7] which we modified. The experiments were performed as follows. The liver, brain, kidneys and lungs were cut up finely with scissors on ice, samples weighing 500 mg of each tissue were transferred to two tubes, 1.5 ml of a phosphate buffer was added to each and half the tubes were placed for 5 minutes in a boiling water bath. After this, to all the tubes were added 1 ml of a 1% solution of thioester, and they were then placed for a definite time in the incubator at a temperature of 37°C. At the end of incubation the quantity of residual unsplit thioester in all the tubes was determined.

Further confirmation of the idea that the preparation is in practice not split up in the body was provided by biological test experiments, based on the fact that a mydriatic effect is a property which is peculiar to the intact thioester molecule. The method of performance of these experiments was as follows. A rabbit was injected intramuscularly with thioester solution, and then after a definite time its urine was extracted by puncture of the bladder, and injected into the eye of another rabbit. In this way a marked mydriatic action was

observed. From a comparison of all these findings it could be concluded with sufficient confidence that thioester undergoes practically no significant degree of decomposition in the body and is excreted in an unchanged form.

It was established that under these experimental conditions enzymic hydrolysis of the preparation does not take place. Incubation even for four hours did not result in any perceptible diminution in the quantity of the preparation added to the tissues. In an experiment using highly purified liver esterase, the whole quantity of preparation added was also detected in its entirety.

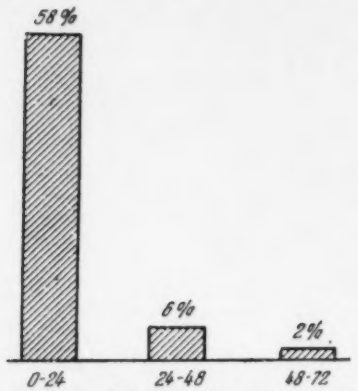


Fig. 2. Excretion of S^{35} by rats after intramuscular injection of thioester.

Experiments in which, besides the distribution, the content of the preparation in the urine was studied, showed that it is present there 5 minutes after injection. The rate of excretion of thioester was studied in special experiments on dogs with ureters transplanted under the skin of the abdominal wall. It was shown that the preparation leaves the body very rapidly, mainly in the urine. The role of the gastrointestinal tract in the process of excretion of thioester was found to be very insignificant.

In order to make a closer study of the process of excretion of the preparation, experiments were set up on rats. In 4 series of experiments the excretion of thioester was investigated 12, 24, 48 and 72 hours after the moment of injection of a 1% aqueous solution of the preparation in a dose of 10 mg/kg (Fig. 2).

During the first 24 hours the urine of the rats was found to contain on the average 58% of the dose injected, during the second 24 hours – 6% and during the third 24 hours – 2% altogether. It was shown that the greater part of the preparation (55%) was excreted by the rats in the first 12 hours. Later still the excretion of the preparation became very slight.

As a result of the work which has been carried out a number of the distinctive features of the distribution of thioester at different periods after its injection have been made clear. It has been established that the preparation rapidly leaves the body, mainly in the urine. These phenomena may be associated with the transient therapeutic action of thioester. The results of the experiments also give grounds for the belief that a considerable part of the preparation is excreted from the body in an unchanged form. One of the future tasks in the field of study of the fate of cholinolytic substances in the body must be to determine the distribution and the rate of excretion of the different preparations of this type with a view to establishing the relationship between the changes in the distribution of the preparations in the body and their chemical structure and physicochemical properties.

SUMMARY

Chlorhydrate of diethylaminoethyl S-ester of diphenyloxythioacetic acid (thioester) demonstrated the specific peculiarities of distribution in intramuscular injection to dogs, cats, rabbits and rats. Its maximal quantities are revealed in the lungs in a short time (up to 1 hour) after the administration of the thioether. The product of hydrolysis of the thioester, the thioalcohol, differs from the thioester by its distribution. The maximal quantitity of thioalcohol is revealed in the kidneys at the same time interval.

Evidently, the thioester does not undergo any significant fermentative disintegration in the animal body and is rapidly excreted in the urine, mostly in unchanged condition. The role of the gastrointestinal tract in the process of thioester excretion is rather insignificant.

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THE DEPENDENCE OF THE LIPOPEXIC FUNCTION OF THE LUNGS ON THE TEMPERATURE OF EXTERNAL ENVIRONMENT

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There is a considerable quantity of experimental data proving that the lungs retain and split fats and oxidize fatty acids [3-7 and others]. The lipopexic function of the lungs is associated with liver activity: the lungs supplement and compensate liver functions in regulating fat metabolism [8]. There is no evidence that along with active participation by the lungs in fat metabolism, they also participate even to the slighest extent in metabolism of other food substances – proteins and carbohydrates [1, 9].

No experimental work devoted to association of lipopexic function of the lungs and temperature of the external environment was found in the literature on the role of lungs in fat metabolism. Observations on seasonal fluctuations of lipids in the blood of healthy man are available. The lipid content of the blood of healthy individuals tends to become lower during the winter months [2, 10, 11]. It can be assumed that the lowering of the lipid content of blood at low temperatures of the external environment is associated with the lipopexic function of the lungs. It is possible that under such conditions fats mobilized from fat depots are transported by the blood to the lungs where their oxidation takes place.

We carried out investigations with the purpose of studying the lipid content of blood depending on the temperature of the external environment.

EXPERIMENTAL METHOD

Experiments were carried out on warm-blooded animals during the summer months of 1955–1956, experimental animals – rats, guinea pigs and cats. All animals were divided into two groups. One group was kept at the temperature of 4-6° for 3-6 days, the other – at the temperature of 25-28°. There were 10 series in these experiments. Each series contained one group of "cold" and one group of "warm" animals. Feeding was the same in both groups. After a period during which one group of animals was kept at a high and the other group at a low temperature, the animals were sacrificed by decapitation and the lungs were removed.

The lungs of 28 "cold" and 29 "warm" rats, of 2 "cold" and 2 "warm" cats and of 2 "cold" and 2 "warm" guinea pigs were subjected to investigation. In such a manner we studied the lungs of 65 animals.

The lungs removed were placed into a drying compartment for 10 hours at a temperature of about 95°. The dried organs were pulverized and again dried to a constant weight. Following cooling in the exsiccator the dry powder from the organs was placed into packages of filter paper which was previously defatted and dried to a constant weight. The weight of the package and the package plus the powder was determined on analytical scales with an accuracy of ±0.2 mg. The weighed packages with powder from "cold" and "warm" lungs were placed into Soksiet's apparatus and subjected to extraction with ether for eight hours. Following extraction of lipids the packages were again placed into a drying compartment for 6 hours at a temperature of about 50° to insure complete evaporation of ether. After cooling in the exsiccator the packages were again weighed with an accuracy of ±0.2 mg. The difference in the weight of the powder before and after extraction

with ether corresponded to the quantity of lipids (in mg) extracted from a given powder. This difference in weight was expressed as a percentage in relation to the initial weight (percentage of loss).

In each series of experiments the average value of the percentage of weight loss in "cold" and "warm" lungs was calculated. To obtain the average of 4 and 5 experiments the average square deviation was calculated using the formula $\sigma = \pm \sqrt{\frac{\epsilon d^2}{n-1}}$, where ϵd^2 is the sum of the squares of the differences between the average and each individual value, and n is the number of calculations.

EXPERIMENTAL RESULTS

Data presented in the table shows that the lungs of animals kept at low temperature revealed a tendency to a somewhat higher content of lipids as compared with lungs of animals kept at a high temperature.

It may be assumed that the temperature of inhaled air influences the lipopexic function of the lungs causing its intensification in the cold.

The Influence of the Temperatures of the External Environment of the Lipid Content of the Lungs of Animals

Series number and animal	Weight of lungs in mg and % of weight loss							
	in the cold			% of loss $(\frac{A-B}{A} 100)$	in the warm			% of loss $(\frac{A-B}{A} 100)$
	animal No.	wt. prior to extraction (B)	wt. following extraction (B)		animal No.	wt. prior to extraction (A)	wt. following extraction (B)	
I (rats)	1	278,0	246,0	11,5	6	210,8	192,8	8,5
	2	339,7	298,3	12,2	7	313,0	287,0	8,3
	3	502,4	442,8	11,8	8	239,5	212,6	11,2
	4	312,9	273,8	12,5	9	380,0	338,0	11,0
	5	393,5	341,5	13,2	10	312,6	288,0	7,9
	Average . .			12,2 $\sigma = \pm 0,66$	Average			9,4 $\sigma = \pm 1,59$
II (rats)	1	253,8	209,6	17,4	3	505,6	443,3	12,3
	2	252,2	214,6	14,9	4	392,0	346,9	11,5
					5	270,9	229,6	15,2
	Average			16,2	Average			13,0
III (rats)	1	246,7	217,8	11,7	5	240,0	215,6	10,2
	2	388,6	302,3	10,7	6	244,1	222,2	9,0
	3	284,1	204,1	9,9	7	291,9	269,6	7,6
	4	359,2	323,0	10,1	8	301,1	275,0	8,7
					9	274,5	247,5	9,8
	Average			10,6 $\sigma = \pm 0,81$	Average			9,1 $\sigma = \pm 0,81$
IV IV (cats)	1	1025,8	881,2	14,1	3	1057,2	934,3	11,6
	2	1064,4	942,0	11,5	4	1215,6	1071,1	11,9
	Average			12,8	Average			11,8

(continued)

Series number and animal	Weight of lungs in mg and % of weight loss							
	in the cold			% of loss $(\frac{A-B}{A}100)$	in the warm			% of loss $(\frac{A-B}{A}100)$
	animal No.	wt. prior to extraction (A)	wt. following extraction (B)		animal No.	wt. prior to extraction (A)	wt. following extraction (B)	
V (rats)	1	181,7	158,3	12,8	5	306,0	269,3	12,0
	2	270,0	234,4	13,2	6	288,2	250,3	13,2
	3	249,3	214,5	13,9	7	282,0	246,3	12,7
	4	296,3	258,5	12,7	8	212,9	184,7	13,2
	Average . . .			13,2 $\sigma=\pm 0,54$	Average . . .			12,8 $\sigma=\pm 0,53$
VI (rats)	1	215,3	197,2	8,4	4	148,3	137,1	7,6
	2	241,3	222,7	7,7	5	255,1	240,9	5,6
	3	186,8	175,6	6,0	6	169,3	159,8	5,6
	Average . . .			7,4	Average . . .			6,3
VII (rats)	1	183,0	168,7	7,8	4	122,9	113,8	7,4
	2	168,6	155,3	7,9	5	190,7	178,1	6,5
	3	204,7	185,8	9,2	6	269,1	256,8	4,6
	Average . . .			8,3	Average . . .			6,2
VIII (rats)	1	311,9	286,4	8,1	5	198,9	183,8	7,6
	2	227,2	210,8	7,2	6	215,7	198,3	8,1
	3	168,8	152,1	9,8	7	241,0	220,8	8,3
	4	103,6	91,4	11,7	8	328,1	298,2	9,1
	Average . . .			9,2 $\sigma=\pm 1,98$	Average . . .			8,3 $\sigma=\pm 0,62$
IX (guinea pigs)	1	641,5	560,6	12,6	3	1318,7	1203,3	8,8
	2	886,2	772,5	12,8	4	781,6	699,1	10,6
	Average . . .			12,7	Average . . .			9,7
X (rats)	1	330,9	293,0	11,5	4	260,9	232,7	10,8
	2	224,3	200,6	10,6	5	272,4	245,0	10,0
	3	265,4	236,9	10,7				
	Average . . .			10,9	Average . .			10,4

SUMMARY

The quantity of lipids was determined in the lungs of rats, cats and guinea pigs. It was demonstrated that there is a slightly increased content of lipids in the lung tissue of animals kept at low temperature.

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*In Russian.

PHARMACOLOGY

THE EFFECT OF A SUBSTANCE WITH THYROID ACTION (IODIZED CASEIN) ON THE COURSE AND OUTCOME OF CORASOLE INTOXICATION IN MICE

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O. D. Kozlov [1] demonstrated that one of the agents disturbing the processes of conjugated phosphorylation (separating respiration from phosphorylation),– 2,4 – dinitrophenol (DNP) – decreases the number of convulsive attacks and lowers the death incidence in animals with corasole intoxication. One of the explanations for these findings linked the effect of DNP with its effect on the processes of conjugated phosphorylation. In order to verify this assumption it was decided to investigate the effect of other inhibitors of respiratory phosphorylation on the course and outcome of corasole intoxication. Thyroxin is such a substance, a substance natural to the organism and done whose separating action was demonstrated by a number of authors in experiments on isolated tissues [4, 5, 6, 7, 8, 9 and others].

EXPERIMENTAL METHOD

Our experiments were carried out on adult male mice weighing from 16 to 22 g. At first, O. D. Kozlov's experiments were performed and his findings of favorable effects of DNP on the course of corasole intoxication confirmed; in 5 mice given DNP (20 γ/g) 15 minutes prior to subcutaneous injection of corasole (80 γ/g) the number of convulsive attacks was 14 instead of 38 as in control mice.

As we did not have thyroxin available, preparations with thyroid action were employed in our experiments. Thyroidin, which according to findings on mice tested for resistance to lowering of atmospheric pressure in the pressure chamber (a generally accepted biological test for thyroidin), was available but found to be ineffective. Therefore a series of experiments was undertaken employing iodized casein (IC) – a preparation producing an action analogous to that of thyroxin.

Two series of experiments were carried out with IC. In the first series of 20 mice a suspension of IC (1 ml containing 4 mg IC) was administered subcutaneously three times (1 ml every other day). The control series of 20 mice received the same number of injections of a casein (C) suspension only. Twenty-four hours following the last injection, 20 mice (10 experimental and 10 controls) were tested for resistance to lowering of atmospheric pressure in the pressure chamber. The remaining 20 mice were given corasole. It was administered subcutaneously in a 1% solution of 80 γ/g. In the second series of experiments IC was given six times.

EXPERIMENTAL RESULTS

As we did not observe any significant difference in the results in both series of experiments, the findings are summarized and presented in the table.

As seen in the table, IC does not influence mortality of mice with corasole intoxication when administered in doses which distinctly decrease resistance of mice to lowering of atmospheric pressure; the number of

fatalities remains the same as in experiments with preliminary administration of uniodized casein (IC does not influence the number of convulsive attacks either).

Thus, of the two separating agents, DNP improves the course and outcome in corasole intoxication whereas the preparation with thyroid action is without any effect.

As is already known, animals with thyrotoxicosis have symptoms resembling those in dinitrophenol and dinitrocresol intoxications: weight loss, rise in body temperature and increased metabolism. On the other hand, as opposed to thyroidin, dinitrocresol cannot eliminate the development of myxedema and DNP does not hasten (as opposed to substances with a thyroid action) metamorphosis of the tadpole.

The Effect of Iodized Casein (IC) on Resistance of Mice to Lowering of Atmospheric Pressure and to Corasole Intoxication

In the pressure chamber				Outside the pressure chamber			
preparation	total number of animals	animals which perished at 270 mm of Hg		preparation	total number of animals	animals perished	
		total number	%			total number	%
C	20	0	0	C + corasole	20	10	50
IC	20	17	85	IC + corasole	20	8	40

There are a number of reports in the literature testifying to the differences in the effect of thyroidin and DNP on the processes of conjugated phosphorylation. The effects on these processes are more distinct when the aid of magnesium salts is enlisted: the magnesium cation inhibits the separating effect of thyroidin but does not influence the effect of DNP [2, 3, 10 and others].

The lack of effect of substances with thyroid action on the course and outcome of corasole intoxication can serve as a new index for differentiating the effects of these substances from DNP. The mechanism of the favorable effect of DNP on the course of corasole intoxication deserves futher study.

SUMMARY

It was demonstrated that the symptoms of corasole intoxication become less intense as a result of preliminary administration of 2,4-dinitrophenol, disturbing the processes connected with phosphorylation. Iodized casein, like substances with thyroid action, disturbs the processes of conjugated phosphorylation, but has no effect on the course and the outcome of corasole intoxication (as distinguished from 2,4-dinitrophenol).

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THE EFFECT OF PHENAMINE ON THE MOTOR EXCITATION CAUSED BY BARBITURATES

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Hyperkinesis caused by barbiturates and especially distinctly expressed by small rodents (mice and rats) can be utilized as an object for the study of different substances capable of raising or lowering the excitability of the central nervous system. Hyperkinesis is manifested in 2 forms: 1) in locomotor excitation associated with depression of the cerebral cortex and 2) in the subsequent tremors depending on the exciting action of barbiturates on the brain stem [midbrain (mesencephalon) and even interbrain (diencephalon)]. The latter form of hyperkinesis was formerly called by us "barbiturate hyperkinesis" [2, 3], and we now perfer to call it "brain-stem hyperkinesis."

In the current project we studied the effect of phenamine on both forms of motor excitation caused in mice by hexenal (hexobarbital).

EXPERIMENTAL METHOD

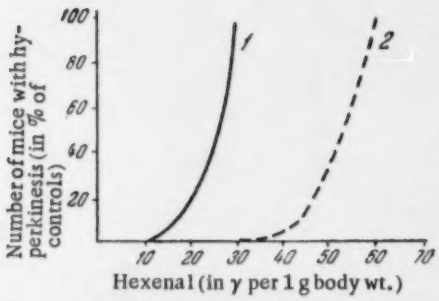
Experiments were carried out on mice of 3 age groups (400 animals): baby mice (weighing 4-5 g), young mice (weighing 10-12 g) and adult mice (weighing 16-18 g). All animals were obtained from one breeding laboratory. The substances studied were administered in aqueous solution subcutaneously in the animal's back. Phenamine was used in doses of 0.1 to 5 γ per 1 g body weight so as not to produce motor excitation in the control animals.

EXPERIMENTAL RESULTS

Preliminary administration of phenamine promoted development of locomotor excitation and brain-stem hyperkinesis from doses of hexenal (15-30 γ per 1 g body weight), which alone, according to our findings, did not produce it (see figure).

During the 6 hours following the administration of phenamine the mice sat quitely in groups within the jars, but when at the end of this interval an optimal dose of hexenal (60-80 γ per g of body weight) was administered the animals entered a state of extreme motor excitation. Especially marked was locomotor excitation and brain-stem hyperkinesis in mice 2-3 weeks of age (weighing 4-5 g). Locomotor excitation was in the form of constant running about (besides, the mice jumped, squeaked, bit one another, etc. Brain-stem hyperkinesis in the animals manifested itself in stronger and more prolonged tremors of the extremities, trunk, head and tail.

In the control group, hexenal (in doses indicated above) produced side reactions in all animals. As is known, phenamine is an antagonist of barbiturates. Therefore, it was not unexpected to observe that side reactions were observed not in all of the experimental animals; brain-stem hyperkinesis manifested itself in them in the form of very quick, small jumps in one place (5 jumps per second, for example) with frequent falling over followed by getting up once more.



Decrease in the dose of hexenal required to produce stem hyperkinesis in adult mice under the effect of phenamine. 1) mice given a preliminary injection of phenamine in the dose of 5 γ per 1 g of body weight (25 animals); 2) mice given hexenal only (25 animals).

It must be pointed out that in mice of different batches (from different breeding laboratories) phenamine may fail to produce motor excitation, even when administered in relatively large doses. Low sensitivity in mice was observed in their experiments in our department by Ts'in Wo-i/Ch'ih chih-ts'ien', they observed a sharp increase in locomotor excitation in mice caused by hexenal (60-80 γ per 1 g body weight) following preliminary administration of phenamine in doses as small as 15-20 γ per 1 g body weight).

The phenomenon of brain-stem hyperkinesis demonstrates clearly the antagonism between phenamine and morphine (or proserine). Administration of morphine in doses of 0.5-2 γ per 1 g body weight (or proserine in doses of 0.01-0.1 γ per 1 g body weight) prevents the development of brain-stem hyperkinesis from hexenal in doses already indicated [1, 2]. But if morphine (or proserine) is administered together with phenamine, the latter prevents the depressing effect of morphine (or proserine) and hyperkinesis develops as usual.

According to the literature, the exciting effect of phenamine spreads to the cortex and brain stem. Inasmuch as phenamine does not produce hyperkinesis originating in the brain stem, it must be concluded that it increases the barbiturate brain-stem hyperkinesis. Earlier we demonstrated that under the influence of small doses of paraldehyde or ether which do not cause motor excitation in mice, brain-stem hyperkinesis is produced by hexenal in doses which are ineffective in control animals. This effect was explained by us as due to disinhibition of motor centers of the brain stem [4]. Increase in motor excitation, produced by hexenal, by the preliminary adminstration of phenamine is sharper than that due to the moderate narcotic action of ether and has a different localization.

SUMMARY

The following was established in experiments on mice. Preliminary administration of phenamine (0.1-5.0 γ per kg of body weight) considerably intensifies the locomotor excitation and the brain stem hyperkinesis caused by hexenal (60-80 γ/g). Hyperkinesis is especially intensified in 2-3-week-old mice weighing 4 to 5 gms. After a preliminary administration of phenamine the locomotor excitation and the hyperkinesis are caused by doses of hexenal (10-30 γ per gm. of weight) which do not cause these phenomena in the control animals. Locomotor excitation caused by hexenal is intensified by the preliminary moderate administration of phenamine much more than by preliminary narcotic action of ether or paraldehyde. Phenamine removes morphine and proserine depression of barbiturate brain-stem hyperkinesis in mice. The effect of phenamine is antagonistic to that of morphine and proserine.

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THE INFLUENCE OF CONGO RED ON THE EFFECTS OF DITILINE

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In 1924 I. R. Petrov was the first to discover the ability of congo red to render curare harmless. This effect was repeatedly confirmed by other investigators [1, 2, 6 and others]. The possibility of rendering curarine isolated from curare harmless was discovered in the last two investigations.

The goal of the current investigation was the clarification of the influence of congo red on the effects of bisquaternary ammonium base – ditiline (dicholine ester of succinic acid), employed in recent years in USSR as a curare-like preparation.

EXPERIMENTAL METHOD

The experimental portion of this project was begun with the performance of experiments on the abdominal rectus muscle of the frog; under the influence of ditiline contracture of the muscle was induced [4]. The isolated muscle was placed in a bath of aerated Ringer solution and 1-2 hours later, twice for each experiment, its reactivity to one or the other concentration of ditiline (1:1 – 2 min) was checked. After repeated changes of the Ringer solution in the glass the latter was filled with a solution containing congo red $1 \cdot 10^{-6} - 1 \cdot 10^{-4}$, and 15-30 minutes later ditiline was added in the concentrations previously tested (a mixture of congo red and ditiline in previously stated concentrations was tested in a series of experiments. Muscle contractions were recorded on the kymograph for 3 minutes with 30 minute intervals between tests.

A total of 15 experiments with 90 tests was performed.

EXPERIMENTAL RESULTS

In experiments with preliminary treatment of the muscle with congo red two types of effects were observed: in smaller concentrations ($1 \cdot 10^{-6}$) congo red increases the contractions of the abdominal rectus muscle caused by ditiline, and in larger concentrations ($1 \cdot 10^{-4}$) suppresses these contractions (Fig. 1).

The second fact revealed itself with great clarity when mixtures of substances studied were tested.

Results obtained on the isolated muscle led on to tests of these substances on the entire organism. Experiments were carried out on 30 male frogs. The substances tested were given subcutaneously: ditiline – in doses of 5 – 10 γ per 1 g of body weight, congo red – in doses of 5 – 100 – 200 γ per 1 g body weight 15-30 minutes prior to administration of ditiline or mixed with it.

Administration of ditiline to frogs resulted in the disappearance of spontaneous movements, contractures of the forelegs, disappearance of the reflex of righting themselves when placed on the back and of the reflex to stimulation of the hindlegs. These effects of ditiline disappeared in 2-3 hours.

All the symptoms caused by ditiline could not be entirely prevented in the majority of the experiments by the administration of congo red. However, congo red did prolong somewhat the latent period preceding the onset of the effects of ditiline and in 18 of 20 experiments prevented the loss of reflexes to stimulation

of the hindlegs of the frog and decreased the intensity of the contracture of the muscles of the forelegs. Prevention of the reflex of righting themselves when placed on the back was more difficult (only in 3 out of 20 cases). Results obtained when congo red and ditiline were administered separately did not differ significantly from those obtained when a mixture of the two was given.

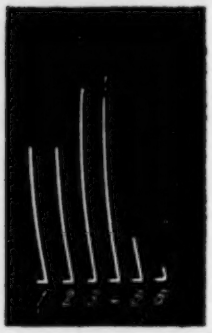

Fig. 1. Contractions of the abdominal rectus muscle of the frog under the influence of ditiline $1 \cdot 10^{-6}$ (experiment No. 13). 1 and 2) Prior to treatment with congo red; 3 and 4) after 30 minutes of treatment with congo red $1 \cdot 10^{-4}$.

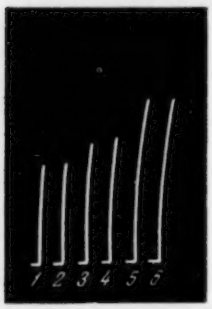

Fig. 2. Contractions of the abdominal rectus muscle of the frog under the influence of acetylcholine $1 \cdot 10^{-7}$ (experiment No. 16). 1 and 2) Prior to treatment with congo red; 3 and 4) after 15 minutes of treatment with congo red $1 \cdot 10^{-6}$; 5 and 6) after 15 minutes of treatment with congo red $1 \cdot 10^{-4}$.

In I. R. Petrov's work [3] which disclosed for the first time the antagonism between congo red and curare and in the investigations of C. J. Kensler [6], one of the last authors studying this problem, the antagonistic effect of congo red in relation to curare (more accurately "rendering it harmless") is explained by the interaction between the dye and the quaternary ammonium base. We studied the possibility of forming a compound between congo red and curarine (curare made by Shuchardt), and also ditiline with the aid of spectrographic analysis: to compare spectrums of individual substances and of their mixtures (for the opportunity to work with the spectrophotometer SF–4 and for the cooperation in the project I declare my appreciation to M. I. Kulenok). Investigations in the visible and ultraviolet fields of the spectrum did not give any grounds for believing that these substances react one with the other to form a new compound.

It is admitted that the antagonistic effect of congo red depends on the action of the dye on the muscle. The possibility of such an influence was assumed even earlier [2].

Interaction between the dye and biological structures also explains the ability of congo red to sensitize the abdominal rectus muscle of the frog to acetylcholine (Fig. 2). This sensitization, apparently, depends on inhibition of cholinesterase by the dye [6]. Apparently, the same explanation holds for the increase in the effect of ditiline (which, like acetylcholine, is a substrate of cholinesterase) which results from small concentrations of congo red.

Thus, interaction between congo red and biological structures (union with prothrombin, complement and cholinesterase) is less controversial than interaction of this dye with quaternary ammonium bases. If the latter does take place, it must lead to formation of a rather unstable compound not detectable by spectrographic analysis in the visible as well as the ultraviolet fields of the spectrum.

SUMMARY

In low concentrations ($1 \cdot 10^{-6}$) congo red intensifies the contractions of the isolated m. rectus abdominis produced by ditiline. Higher concentrations ($1 \cdot 10^{-4}$) of congo red depress these contractions. The first effect is probably connected with the depression of cholinesterase, and the second – with the action of the dye on the muscle. The formation of a congo red compound with the quaternary ammonium bases – ditiline (dicholine ester of succinic acid) and curarine (contained in curare), as suggested in pertinent literature, could not be established by means of a spectrographic analysis.

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ONCOLOGY

EFFECT OF ANTITUMOR SERUMS ON THE GROWTH OF BROWN-PEARCE RABBIT TUMOR IN TISSUE CULTURE

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The explantation method has been applied to the study of the effect of antitumor serums on the growths of tumor cells since the third decade of the present century. The results of such studies have, however, been very conflicting.

Some authors [8, 9] were unable to detect any differences in the growth in tissue culture of various tumor strains in serum taken from animals bearing tumors which were in process of resorption, or which were growing actively. Other workers [1, 7] arrived at similar results using serum from animals which had been immunized against tumor tissue. On the basis of these observations, these workers either denied the existence of antitumor antibodies in general, or else they admitted their existence, but did not attribute any antitumor properties to them.

Recent papers have shown conclusively [2, 3, 4, 6] that antibodies present in the serums of immunized animals are able to inhibit growth of tumor tissue in vitro. It is not yet known, however, whether this inhibitory effect is due to specific or unspecific antibodies.

The present paper presents the results of a comparative study of the effects of a highly specific adsorbed antitumor goat serum, and of the native serum, on the growth in tissue culture of metastatic nodes of Brown-Pearce rabbit tumor.

EXPERIMENTAL METHODS

The immune serums were obtained by ummunizing goats with 10% aqueous saline extracts of tumor tissue. In order to eliminate unspecific antibodies, and to enhance its specificity, some of the serum so obtained was purified by adsorption on the antigen with formalin-treated kidney and spleen tissues from a normal rabbit. The adsorption procedure was described by us in an earlier publication [5].

The serological examination of our serums was effected by means of the complement-fixation test, at 37°. We used three types of goat serum in our experiments: highly specific immune goat serum, treated by the adsorption procedure, containing basically antitumor antibodies, at a titer of 1:320++; native serum, containing both antitumor antibodies (1:640++) and antibodies to normal organs (1:200++); and, as a control, normal goat serum.

Material from metastatic nodes of Brown-Pearce rabbit tumor was grown in tissue culture in Carrel vessels, diameter 3.5 cm. Fowl plasma was first placed aseptically into the vessels, at a rate of two drops per fragment of tissue. The comminuted fragments of tumor tissue were then transferred to the cups by means of a special needle. Six fragments were placed into each cup. Chick embryo extract was then added. After the plasma had clotted, we added 10 drops of the given serum, and 8 drops of saline solution.

We assessed the action of the various serums on growth of tumor tissue on the basis of the number of

fragments showing growth, and of the intensity of growth of each fragment. The intensity of growth of each fragment was evaluated from the ratio of the area of the growth zone to the initial area. The initial area, and that of the growth zone, were measured by tracing the outlines on millimeter paper, using a microscope and an Ra-1 copying device, and counting the number of square millimeters included in the outlines.

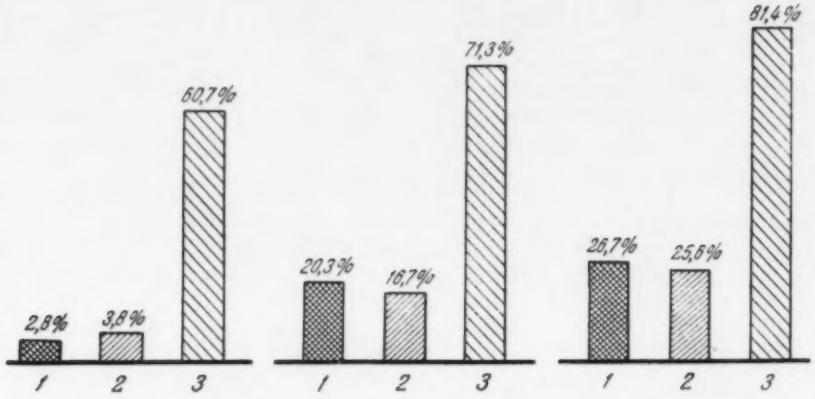

Fig. 1. Growth of tissue cultures of Brown-Pearce rabbit tumor in immune and normal goat serums (area of the growth zone as % of the initial area of the fragment of tissue). On the 5th, 10th, and 15th day of culture: 1) in adsorbed highly specific serum (158 fragments of tissue); 2) in polyspecific serum (174 fragments); 3) in normal goat serum (160 fragments).

EXPERIMENTAL RESULTS

Our results are illustrated in Fig. 1, where they are expressed as percentages on the 5th, 10th, and 15th days.

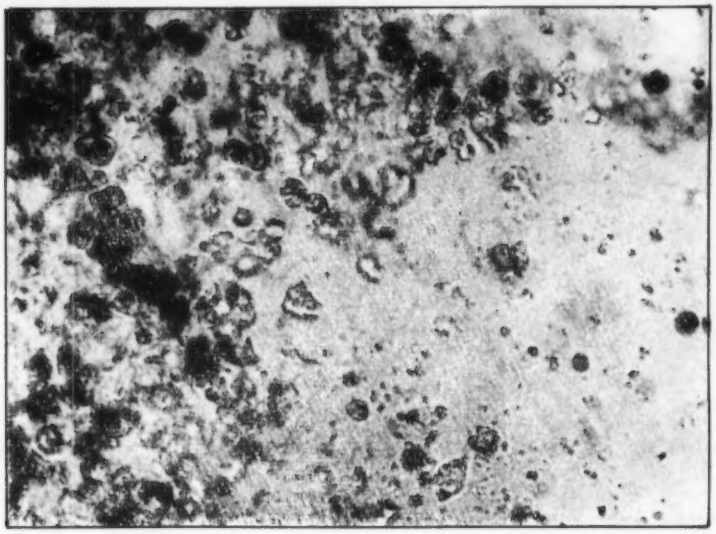

Fig. 2. Growth of tissue from a metastatic node in native immune goat serum (15th day of culture). Magnification 268 ×.

As is evident from Fig. 1, the highest percentage growth of the growing fragments was found in nutrient medium containing normal goat serum (81.4%), on the 15th day. The least percentage growth in this time

was found in media containing native (untreated) goat immune serum (25.6%). Growth in treated goat immune serum amounted to 26.7%. The small difference in growth (1.1%) between media containing treated and untreated serums is not statistically significant.

The rate of growth, up to the 15th day, was greatest in medium containing normal goat serum. In a nutrient medium containing "adsorbed" serum the growth coefficient was 0.18, and with untreated immune serum it was 0.15. The small difference between the growth coefficients (0.03) in the two media is similarly not significant.

The results of our experiments (we used 492 fragments of tumor tissue for explantation, in all) thus showed that explants in nutrient media containing treated or untreated immune sera either did not grow at all, or they grew much more slowly than in media containing normal goat serum, and total or partial destruction of the cells of the explant had taken place by the 15th day of culture (Fig. 2).

The greatest growth in tissue culture of Brown-Pearce rabbit tumor was found in media containing normal goat serum. In such media we observed growth in the form of a unicellular layer, in which we could readily distinguish polymorphous cells with large, clear nuclei, situated very close to each other (Fig. 3).

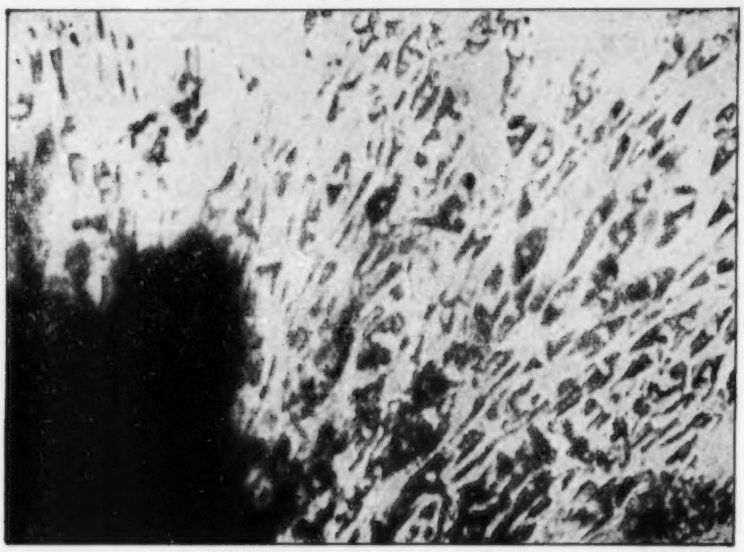

Fig. 3. Growth of tissue from a metastatic node in normal goat serum (12th day of culture). Magnification 268 ×.

We concluded from our findings that serums containing antitumor antibodies powerfully inhibited growth of explants of Brown-Pearce rabbit tumor.

Comparison of the number of explants which showed growth, and of their rate of growth in media containing "adsorbed" and untreated serums showed that they inhibited growth to an equal extent.

Our experimental results, as well as those of other authors [1, 2], justify the conclusion that inhibition of growth of tissues of metastatic nodes of Brown-Pearce rabbit tumor is ascribable to the action of specific antitumor antibodies.

SUMMARY

Growth of Brown-Pearce rabbit tumor in tissue culture is powerfully inhibited by serum from goats which had been immunized against this tumor. Removal from the immune serum of unspecific organ antibodies, by adsorption on formalin-treated rabbit tissues, gave a highly specific antitumor serum, which inhibited growth of explants to the same extent as did the untreated serum. It is concluded that the inhibitory effect is due to the presence of specific antitumor antibodies.

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*Original Russian pagination. See C. B. Translation.

EFFECT OF SPECIFIC VACCINATION ON METASTASIS OF THE BROWN-PEARCE RABBIT TUMOR

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Many aspects of the problem of specific antitumor vaccination still await elucidation. There is conclusive evidence of the possibility of prophylactic vaccination against transplanted tumors, effected by resorption of living tumor tissue. It is not clear whether the vaccinatory effect of resorption of living tumor cells applies to tumors already present in the organism. The great majority of researches designed to test this possibility gave negative results [1, 7], from which a number of workers have drawn the conclusion that therapeutic vaccination is not applicable to transplanted, and a fortiori to spontaneously arising tumors [12, 8]. At the present time, however, advances made in the study of immunity to malignant tumors have established the theoretical possibility of therapeutic vaccination, and have delineated the experimental approach towards its achievement [4, 2, 9]. There are only a few reports of the successful application of vaccinotherapy to the treatment of tumors, mostly published during the past few years [10, 11, 5, 6]. From these reports [5] it appears that the therapeutic effect of resorption of the vaccinating tumor is evident only when this takes place at an early stage of development of the original tumor.

The object of the present research was to investigate the therapeutic effect of vaccination on postoperative development of metastatic tumors. We emerged with the assumption [4] that vaccinotherapy might be more effective against metastases after excision of the primary tumor than when it had not been removed. We were unable to trace any literature references to any experimental work of this sort.

EXPERIMENTAL METHODS

Our experiments were performed on rabbits bearing the readily metastasizing Brown-Pearce tumor. We used 40 chinchilla bucks, of an average weight of 2 kg. Vaccination was effected by introducing virulent tumor tissue under the skin of an ear [3]. The advantage of this procedure was that it assured regular and rapid resorption of the inoculum. Vaccination was effected by a single subcutaneous injection of 0.26-0.27 ml of a 40% suspension of comminuted tumor tissue in physiological saline. The primary tumor was produced by inoculation into the right testicle of rabbits of 0.75 ml of a 20% suspension of fresh tumor tissue.

The experiments were designed to show at what stage of the tumor process vaccinotherapy would be effective. The animals were accordingly divided into 4 groups, of 10 rabbits each. In the first group vaccination was performed 13 days before implantation of the tumor into the testicle. The rabbits of the second group were vaccinated simultaneously with implantation of the tumor. Those of the third group were vaccinated on the 8th day after implantation of the tumor, and one day after its surgical excision. The animals of the fourth group served as controls, and were not vaccinated.

Excision of the testicle containing the implanted tumor was performed on all the experimental and control animals at the same time, on the 7th day after implantation. Observation of the growth of the primary

tumors was continued for 3 months, after which the surviving rabbits were killed. The effectiveness of vaccinotherapy was assessed from the number of successful implants into the testes, and from the incidence and extent of metastases after excision of the primary tumor.

EXPERIMENTAL RESULTS

Macroscopic observation showed that in the first group of animals the tumor inocula deposited subcutaneously in the pinna at first grew, and then, by the 13-18th day, underwent resorption, in all 10 animals. Similar effects were seen in 8 out of 9 rabbits of the second group, although the process of resorption of the vaccinating inoculum was more prolonged (Fig. 1). In one rabbit (No. 3265) the tumor continued to grow beneath the skin of the ear for a long time, and had not yet undergone resorption when the animal died of the primary tumor. The development of the vaccinating tumor in the third group differed from that in the first and second groups (Fig. 2). As is evident from Fig. 2, the vaccinating tumor either showed no growth at all, or grew only very slowly, in 7 of the rabbits, while in 3 of them (Nos. 1874, 2937, and 3030) its development took a very chronic course, resorption beginning very late, and achieving completion only in one case (rabbit No. 3030), by which time the other two rabbits had already succumbed to the primary malignant process. It thus appears that inoculation under the skin of the ear of an animal at an advanced stage of development of the primary tumor is either not followed by growth of the vaccinating tumor, or is followed by its retarded development.

The effects of vaccination on the course of the primary malignant process are shown in the table and in Fig. 3.

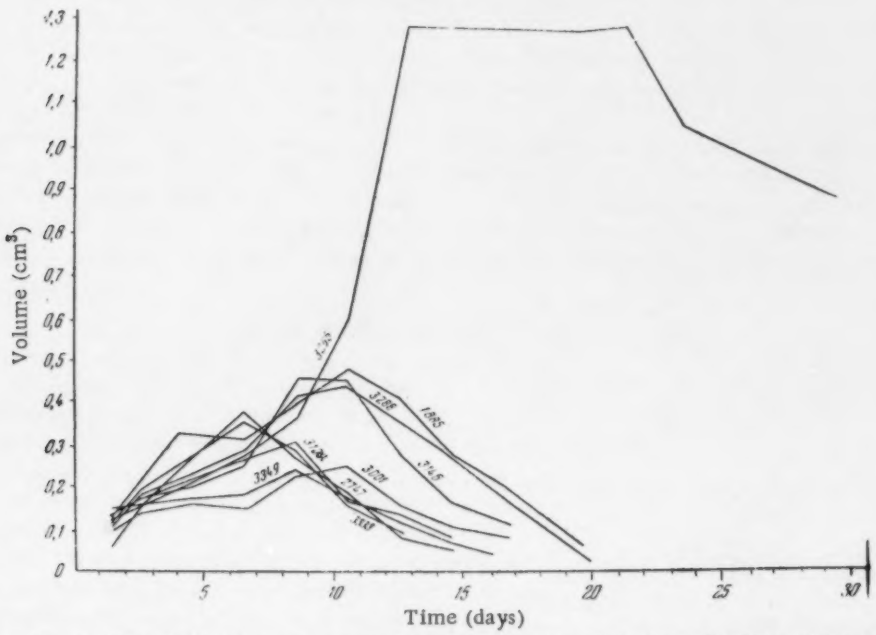

Fig. 1. Growth of vaccinating tumors beneath the skin of the ears of rabbits of the second group of experiments. Curves showing variations in volume of the tumors with time after inoculation.

These data show that, as was to be expected, the animals of the first group differed from those of the other groups; the implants into the testes did not take. Considerable growth of the primary implants took place in all the animals of the second, third, and control groups, as was shown by observations made before excision of the affected testicles, and by histological examination of the excised tissues; the nature and rate of growth of the tumors were the same in these three groups.

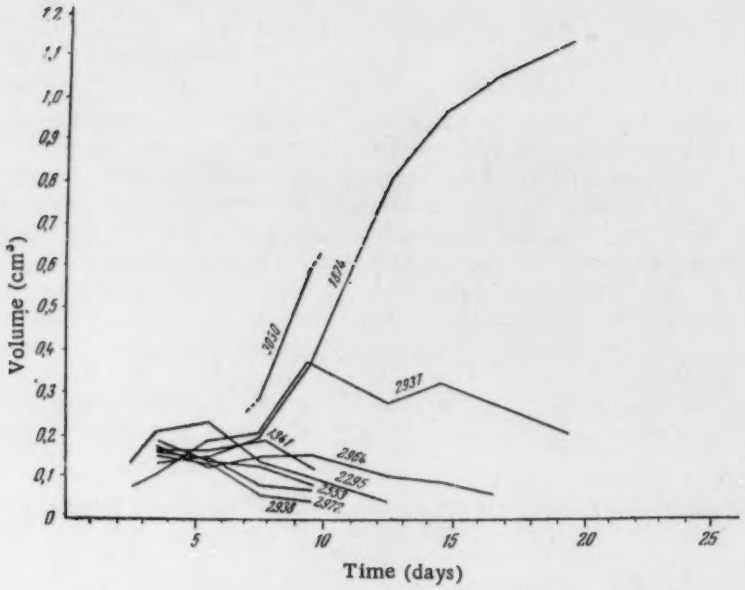

Fig. 2. Development of vaccinating tumors beneath the skin of the ears of rabbits of the third group of experiments. Curves showing variations in volume of the tumors with time after inoculation. Measurement of the volume of the tumor of rabbit No. 3030 was discontinued, for technical reasons.

The evolution of the cancerous process after excision of the testicles proceeded differently in the various groups of animals. The animals of the first group showed no signs of tumor development over a period of 3 months of observation. Anatomopathological examination showed the absence of metastases in 7 of the animals of this group (70%). Solitary, very small metastases were found in some of the organs of the remaining 3 rabbits of this group; histological examination showed that these metastases were in process of encapsulation and resorption.

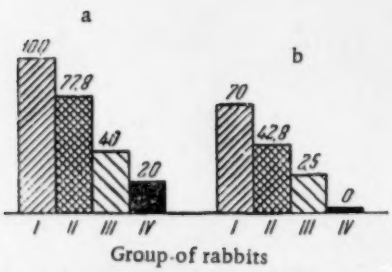

Fig. 3. Mortality and morbidity data for the various groups of experimental animals, over a 3 month period of observation: a) % survival rate; b) percentage of survivors not showing metastases.

In contrast to the animals of the first group, the animals of the second, third, and control groups showed certain changes, which were initially the same for all these groups, in the vicinity of the excised tumor-bearing testicle. These consisted in the appearance in the scrotum and in the spermatic cord of small, dense nodules. The subsequent course of the process varied, however, for the different groups. In the second group these nodules had disappeared by the 15-16th day in 7 rabbits (77.8%), by which time resorption of the vaccinating tumor was almost completed, and these animals subsequently gave no evidence of the continued existence of the neoplastic process. Metastases were not found at autopsy in 3 rabbits (42.8%). Metastases were seen in 4 animals, but they were solitary and small, were found in only 1 or 2 organs, and were in process of encapsulation and resorption. In this group 2 rabbits died owing to the metastases (Nos. 3265 and 3349). Widespread metastases were found in 8 of 10 rabbits of group III, causing the death of 6 animals. In two of the rabbits the metastases began to regress after $1\frac{1}{2}$ -2 months of progressive growth, as shown by observations made on the live animals. The neoplastic process took a benign course in the remaining two rabbits, from the time of implantation. In the four surviving animals

Effect of Vaccination on Development of the Primary Tumor

Group of rabbits	No. of rabbits	Take of tumor implant in the testicle		Development of metastases over a 3 month period								
				rabbits died				rabbits survived				
		did not take	took	number	mean survival time	average No. of metastases	average No. of organs metastasized	number	rabbits without metastases	rabbits with metastases	average No. of metastases	average No. of organs metastasized
First: vaccinated before implantation of tumor in a testicle	10	10	–	–	–	–	–	10	7	3	4	2
Second: vaccinated simultaneously with implantation of tumor	9	–	9	2	33.5	263	10	7	3	4	18.2	1.5
Third: vaccinated after implantation of tumor in a testicle	10	–	10	6	38	715.6	9.5	4	1	3	24.3	4.7
Fourth: control	10	–	10	8	35.7	675.5	10	2	–	2	43.5	5

(40%) anatomopathological examination did not reveal any metastases in one, and those in the other three were in process of resorption. Metastases were found in all the animals of the control group, of which 8 died during the period of observation, and 2 survived (20%). Metastases were also present in these two rabbits, although most of them had undergone resorption.

The following conclusion may be drawn from our results. Vaccination of rabbits by means of a single implantation of Brown-Pearce tumor tissue under the skin of an ear, performed simultaneously with implantation of the same tumor into a testicle, prevents formation of metastases developing after subsequent excision of the testicular implant, in most of the animals. Vaccination performed at this stage is, however, less effective than is prophylactic vaccination. Vaccination performed on the 8th day after implantation into the testicle, and one day after its excision, has little effect, and metastases develop in most of the animals.

A comparison of the outcome of the primary neoplastic process with the course of development of the vaccinating tumor in the same rabbit shows that a therapeutic effect is found in those cases in which the vaccinating tumor has had time to grow actively, and to begin to regress, in the early stages of the metastatic process. Where this is not the case, the primary neoplastic process may cause the death of the animal before regression of the vaccinating tumor is completed, and it may, moreover, interfere with the development of the vaccinating tumor, so adversely affecting its immunizing effect.

We believe that our experiments show the possibility of applying vaccinotherapy to the treatment of metastases developing after excision of a primary tumor, and that they indicate some of the conditions necessary for the achievement of this effect. Further researches in this direction are essential.

SUMMARY

Rabbits were vaccinated by subcutaneous implantation of living Brown-Pearce tumor cells into a pinna, before, at the same time as, and after implantation of the same tumor into a testicle, which was excised, together with the implant, after 7 days.

Subsequent development of metastases did not occur, or was greatly reduced, in animals vaccinated before or at the same time as implantation into the testicle. Only a negligible effect of vaccination was found in animals vaccinated 8 days after implantation. It is concluded that vaccinotherapy is only effective when the vaccinating tumor has time to regress before the metastatic process has reached an advanced stage.

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EXPERIMENTAL TUMORS INDUCED IN RATS BY DICHLOROBENZIDINE

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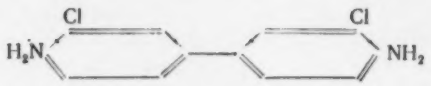
Benzidine and certain other polycyclic diamines are important intermediates in the production of synthetic dyes.

It is known that benzidine is a carcinogenic substance, and that to its action are due numerous cases of cancer of the bladder of workers in the aniline dye industry [1, 3, 5, 6, 9]. There are numerous references in the world literature to the blastomatogenic action of some benzidine derivatives (dianisidine, o-tolidine). Some authors believe that dianisidine may cause tumors of the urinary bladder [1, 10]. Since, however, the same individuals who are exposed professionally to dianisidine are also exposed to benzidine, there is as yet no conclusive evidence of the carcinogenic action of dianisidine. As for o-tolidine, this is not usually considered to be a carcinogen, although in experiments on rats it has been shown to cause tumors of the Zymbal glands [11] in 4.3% of cases.

No data have been published on the carcinogenic properties of dichlorobenzidine (DCB). It is known that DCB can be fairly readily absorbed through the undamaged skin, in particular under conditions of high environmental temperature and humidity [4, 7].

Physicochemical properties. 3,3'-dichlorobenzidine (3,3'-dichloro-4,4'-diaminodiphenyl) is a grey, odorless powder. It is sparingly soluble in water (100 g of water will dissolve 0.07 g of the substance at 15°).



EXPERIMENTAL METHODS

We used 111 sexually mature white rats, weight 110-130 g, in our experiments. The percentage incidence of spontaneous tumors in these rats is extraordinarily low. Only a few tumors were observed, these being sarcoma of the mesentery, and mammary gland tumors in aged virgin females. As controls, we took a group of 130 rats, which had over a period of 10 months received injections of octadecylamine or methylstearylamine into the subcutaneous areolar tissue. No tumors were found in this group over a period of 20 months of observation.

The animals were on an unrestricted diet. The diet was adequate with respect to animals proteins, carbohydrates, and vitamins. The DCB was supplied by the Voroshilov Scientific Research Institute of Organic Intermediates and Dyestuffs. The product was a paste, consisting of 45.3 parts of the substance to 50 parts of water. It was administered either subcutaneously, as a suspension in glycerol or sunflower seed oil, or per os. The organs of all animals dying or killed were subjected to macro- and microscopic examination.

Results of Experiments on Introduction of DCB, and Description of the Tumors

Mode of administration of DCB	Number of animals	Duration of administration of DCB (in mo.)	Total amt. of DCB administered (in grams)	Survived to appearance of the first tumor	Number of tumor-bearing animals	Situation of tumor: Zymbal's gland	Situation of tumor: mammary gland	Situation of tumor: connective tissue and muscles at the injection site	Situation of tumor: connective tissue and muscles "distant"	Situation of tumor: liver	Situation of tumor: hemopoietic system	Situation of tumor: thyroid	Situation of tumor: urinary bladder	Remarks
Fed	50	12	4,53	29	12	2	4	—	2	1	3	1	2	3 rats had 2 tumors each
Injected	61	10—11	1,62—3*	35	18	9	7	5	—	1	2	1	—	4 rats had 2 tumors each, and one had 4 tumors
Total	111			64	30	11	11	5	2	2	5	2	2	

* The reason for the wide variations in dosage is that at the beginning of the experiment one group of rats was injected twice weekly with doses of 60 mg each.

Feeding of DCB. Fifty rats were taken for these experiments (see Table). From 0.5 to 1 ml of a 4.4% suspension of DCB in sunflower seed oil were added to the daily rations, except on Sundays and holidays, for a period of 12 months.

EXPERIMENTAL RESULTS

Acute enlargement of the spleen and liver of one rat was observed, over a period of 10 months from the beginning of the experiments. Microscopic examination of the organs showed the presence of myeloid leucosis.

After 11 months a mammary gland tumor was found in another rat. In all, 12 animals of this group developed tumors. These were variously situated, and 3 of the animals had two different types of tumor (sarcoma of subcutaneous connective tissue with a mammary gland tumor, a thyroid tumor with a hepatoma, a polymorphous cell sarcoma of the wall of a parasitary cyst with a papilloma of the urinary bladder).

Two of the bladder tumors were benign papillomas. One papilloma showed the beginnings of invasive growth into the submucosal layer. The papillomas were found 15 and 16½ months after the start of the experiments.

Tumors of the Zymbal glands (sebaceous glands of the external auditory canal [2]) had an organoid structure, with a preponderance of keratinized squamous tumor cells. The mammary tumors all had the structure of cystoadenocarcinomas; the liver tumors were hepatomas.

Injections of DCB. We used 61 rats for these experiments (see Table). From 20 to 120 mg of DCB were injected dialy into the subcutaneous connective tissue, for a period of 10-11 months. The injections frequently caused an excited state of the animals, with rigors of short duration. Because of the high mortality encountered, the dosage levels were later reduced, and, beginning from the 6th month of the experiment, the rats were given an injection of 0.5 ml of 8.8% DCB emulsion in glycerol once weekly, i.e. 20 mg per week.

A mammary gland tumor was found in one of the rats after 7 months of the injections. A second tumor, of the Zymbal glands, appeared in this same rat 5½ months after surgical removal of the mammary tumor.

In this series of experiments we found tumors in 18 rats, both at the site of injection and in various

internal organs. We shall describe some of the general features of these tumors, without entering into a detailed account of the histology of all the tumors. Multiple tumors were found in 5 rats (2 each in four, and 4 in the fifth rat). These were diagnosed as follows: a rhabdoblastoma at the injection site with a keratinized squamous cell carcinoma of Zymbal's glands; a hepatoma with a keratinized squamous cell carcinoma of Zymbal's gland; carcinomas of Zymbal's gland on the right and the left sides; a mammary adenocarcinoma with a Zymbal's gland carcinoma; a cystoadenocarcinoma and 2 fibroadenomas of the mammary gland with a thyroid adenoma. The Zymbal's gland tumors mostly had an organoid structure, and had in some cases caused erosion of the facial bone. The majority of the mammary tumors were cystoadenocarcinomas, with patches of consolidation.

We thus found that, of 64 rats which survived to the appearance of the first tumor, 30 (i.e. 47% of cases) developed tumors at various sites and of various morphological structure. In 5 rats tumors appeared not only at the injection sites but also in internal organs. Four of the tumors were sarcomas of different forms, and one was a rhabdomyoblastoma. It may be inferred from our findings that DCB is a relatively potent blastomatogenic agent. In considering the nature of the blastomatogenic action of this substance it is necessary to bear in mind the scanty information on the metabolism of DCB available in the literature. According to Meigs [8] DCB is excreted unchanged in the urine.

On the other hand, animal experiments have shown that it appears in the urine of rats exclusively in the form of metabolites.

Since DCB caused the appearance of tumors not only at the injection site and in parenchymatous organs, but also in the urinary bladder, where, according to the findings of our laboratory, it is not present, it may be supposed that the blastomatogenic effect is exerted both by dichlorobenzidine and by its metabolites.

Per os or subcutaneous administration of dichlorobenzidine to rats caused the appearance of tumors in a high proportion of cases. The tumors appearing after subcutaneous injection of DCB were situated not only at the injection site, but also in internal organs.

SUMMARY

Prolonged administration of dichlorobenzidine, per os, or by injection into the subcutaneous connective tissue led to appearance of tumors in a substantial proportion of cases. Of the original number of 111 white rats, 64 survived to the appearance of the first tumor, and of these rats 30 had tumors of various types of locations, including 5 sarcomas at the injection site. It appears that both dichlorobenzidine and its metabolites possess cancerogenic activity in the rat.

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EXPERIMENTAL BIOLOGY

RELATION BETWEEN DEVELOPMENT OF FUNCTION AND STRUCTURE OF THE CEREBRAL CORTEX IN ONTOGENESIS

PART III. ON CERTAIN FINE HISTOLOGICAL STRUCTURES PARTICIPATING IN THE ACCOMPLISHMENT OF THE FUNCTION OF CLOSING (OF REFLEX ARCS)

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Physiological and histological data on the ontogenesis of the cerebral cortex of rabbits were presented in our preceding communications [4, 5].

In the present paper, the ages at which conditioned reflexes can be elaborated are considered in conjunction with histological data, with the object of ascertaining what histological picture corresponds with the state of functional maturity of the cortical cells.

It should be noted that the establishment of a correlation between function and structure, based on their synchronous appearance in the course of ontogenesis, does not constitute direct evidence that these events are casually interrelated. It may never the less provide some indications as to the significance of certain histological structures for the accomplishment of (reflex arc) closing functions, especially if the correlations established prove to be applicable to a number of analyzers.

For convenience, the physiological and the histological data are presented side by side in the Table.*

It is of interest, for the establishment of any correlations between physiological and histological data, to establish the existence of a connexion between the origination of any function and the appearance of certain new structures, i.e. the achievement of a definite stage in the development of the neuron. Only in such a case is it possible to attribute a function to a given structure, and the conclusions drawn assume a general nature. Conclusions as to the significance of cell structures are more conveniently derived separately for the structure of the cytoplasm, and separately for the cell processes with their synaptic apparatuses.

In making the comparisons, it should be borne in mind that olfactory stimuli are projected to the region of the rhinencephalon, whereas tactile, auditory, and visual stimuli are projected to various regions of the neencephalon.

We shall first consider which internal cytoplasmic structures of nerve cells of the cerebral cortex are essential for the formation of a conditioned reflex. We shall begin with the neencephalon, the development of which is completed during extra-uterine life. By the age of 10 days, when conditioned reflexes to auditory and tactile stimuli first begin to make their appearance, we first began to find, in all the layers of the

*The data relating to the histological differentiation of the cells are presented in a shotened form. More complete data were recorded in Part II of this series (Tables 1 and 2) [5].

Comparison of the Results of Physiological and Histological Study of the Cortex and Subcortical Region of Rabbit Brain, at Different Stages of Postnatal Development

Age, in days after birth	Physiological data	Histological data: structure of cytoplasm							Histological data: structure of processes and synapses			
		subcortex cent. high unconditioned reflexes	olfact. cortex – olfactory analyzer	neencephalon (skin, auditory, visual, and motor analyzers) layer II	layer III	layer IV	layer V	layer VI	subcortex	olfactory cortex	neencephalon layer III	neencephalon layer V
0—1	Unconditioned reflexes											
	Conditioned food reflexes to smell	3	3,2	1 (2)	1 (2)	1	2	2,1	III	III	1(IIa)	IIa
5—6	Ditto	3	3	2 (1)	2	1 (2)	2 (3)	2	III	III	IIa	IIa
8	» »	3	3	2 (1)	2 (1,3)	1 (2)	3 (2)	3 (2)	—	—	—	—
10	The same, and unstable conditioned reflexes to sound, touch, and heat	3	3	2 (1,3)	2,3	1,2(single cells 3) visual region 1,2	3	3 (2)	III	III	II a	II b III
13	The same, and more stable conditioned reflexes to the specified stimuli	3	3	3 (2)	3	3 (2)	3	3	—	—	—	—
	Conditioned reflexes to light											
15—16	Ditto	3	3	3	3	3 (2)	3	3	III	III	III	III
Adult animals	» »	3	3	3	3	3	3	3	III	III	III	III

Designations: Arabic numerals – stages of differentiation (see footnote on p. 1241); Roman numerals – stages of differentiation of cell processes, the figures in parentheses indicating the stage reached by the minority of the cells; IIa) synaptic knobs at the basal parts of the dendrites; IIb) synaptic knobs on the terminal ramifications of the dendrites; III) large numbers of knobs on the dendrites.

neencephalon, cells which are at the third developmental stage (mature nerve cells).* At an earlier age, for example in an 8-day-old rabbit, stage 3 cells are absent from layers II and IV of the cortex. It has been shown that formation of conditioned reflexes is not possible at this age. It is hence natural to associate functioning of the cell with the 3rd stage of development. This assumption is confirmed by the results of study of a number of analyzers, and this largely excludes the possibility of a fortuitous coincidence of the times of functional maturation and of the cells entering into the 3rd stage of differentiation.

Third stage cells are absent from the occipital cortex of a 10-day-old rabbit, and it has accordingly not been found possible to form reflexes to light at this age. Reflexes to visual stimuli can first be formed at the age of 13 days, by which time mature nerve cells are to be found in all the layers of the occipital cortex.

Most of the nerve cells of the rhinencephalon and of the subcortical areas are mature at birth. Accordingly, if the assumption that cell function is contingent on the cells having achieved the third stage of maturity is justified, these parts of the brain should be functional from birth. Physiological experiments have, in fact, shown that the formation of conditioned reflexes to olfactory stimuli projected to the olfactory cortex is possible from the moment of birth, as is the accomplishment of unconditioned reflexes (subcortical region).

Thus the appearance of the closing function can be associated only with the third stage of maturation of the cells. Cells which are at the second, and a fortiori at the first, stage of maturity cannot be regarded as being fully functional. The functioning of the neencephalon requires the presence of mature nerve cells, even though in small numbers, in all the layers of the cortex. Admittedly, in such cases the conditioned reflexes lack stability, as is exemplified in 10-day-old rabbits. As the number of cells achieving maturity increases, however, the conditioned reflexes become more stable.

Thus we find full consonance between the physiological and the histological data.

With which structural changes in the cell may we associate development of function? The most important qualitative change concerned with transition of the cells to the third stage of maturity is the formation of Nissl bodies in the cytoplasm. Thus the most important change in the structure of the neuroplasm is the appearance of Nissl bodies, on which the function of the cells depends. It may hence be assumed that the Nissl bodies are the morphological formations directly concerned with the specific function of the cells.

The application of histochemical methods permits of a somewhat more profound approach to the nature of the processes taking place within nerve cells during their maturation. We have observed [5] that the total ribonucleic acid content of the cells increases considerably during their maturation. This must point to the importance of the part played by ribonucleic acid in the accomplishment of its function by the mature cell. However, the mere presence of ribonucleic acid in the cytoplasm is not in itself sufficient to ensure the development of cell function. It is essential that the ribonucleic acid should enter into the composition of special cytoplasmic structures, represented by the Nissl bodies. Ribonucleic acid, which is present in high concentration in the cytoplasm of second stage cells, fulfills a different role from that in third stage cells; it appears to be concerned in the synthesis of protein for formation of the growing axon. This hypothesis is in agreement with the findings of a number of foreign authors [9]. The direct proportionality between the duration of the second stage of maturation and the length of the axon (Part II) also supports this hypothesis.

The function of the ribonucleic acid changes when the cells have achieved the third stage of maturity, and it now takes part in metabolic processes connected with specific nerve function.

It might, from our general knowledge of the role of ribonucleic acid in living organisms, be supposed that a characteristic feature of the specific processes of nervous activity is the high rate of protein turnover.

Apart from the appearance of Nissl bodies, a second important histological change associated with entry of the cells into the third stage of maturity is the development of the large, differentiated nucleolus, rich in ribonucleic acid. The conclusion may hence be drawn that the nucleolus plays an important part in the accomplishment of cell functions, and this would be in accordance with the findings of a number of authors [10].

*First stage – dark nucleus and clear cytoplasm; second stage – clarification of nucleus, increase in size of nucleolus, accumulation of ribonucleic acid in the cytoplasm; third stage – clear nucleus with a large, differentiated nucleolus, formation of clumps of tigroid in the cytoplasm (Part II [5]).

Comparison of physiological data with those on the development of synaptic apparatuses of cortical cells (see Table) shows that the appearance of the closing function coincides in time with the appearance of bottons on the terminal ramifications of the apical dendrites of the cortical cells. At the age of 10 days, when the number of such bottons is small (stage IIb), conditioned reflexes to stimuli projected to the neencephalon are only formed after a large number of associations, and are of low stability. With increase in the number of bottons (stage III) conditioned reflexes are formed more rapidly, and are more stable. Conditioned reflexes are not formed before the synaptic knobs develop (first 10 days of life). Conditioned reflexes to olfactory stimuli are formed from the first day after birth, and this corresponds with the presence of synaptic knobs on the dendrites of the cells of the rhinencephalon of a new-born rabbit. Synaptic knobs are the morphological substrate of the axodendritic synaptic links [8], and appear to be essential for the formation of the temporal link. The coincidence in time of the origination of the closing function and the formation of bottons on the terminal ramifications of dendrites is also understandable, since the study of the cytoarchitectonics of the cortex has shown that it is in the external layer that the most favorable conditions for establishment of contact between cells are to be found. It may be supposed that closing of the temporal link requires the active participation of the axodendritic synaptic links of the marginal layer of the cortex.

Our data on the times of maturation of the cerebral cortex, obtained by physiological and histological methods, are in agreement with those of other authors, who used other methods for the study of this question: biochemical [6], electrophysiological [1, 3], and other methods, and with the findings of A. A. Volokhov [2] and G. A. Obraztsova [7] as to the times of formation of the twitching conditioned reflex to sound.

We conclude from the results of our experiments that the presence within the cortical cells of the following histological structures is essential for their functioning: 1) formed Nissl bodies; 2) differentiated nucleoli; 3) synaptic knobs on the terminal ramifications of dendrites. The presence of these formations is evidence of the functional maturity of the cells.

In associating the given structures with the possibility of accomplishing the closing function we do not, of course, claim that the mere presence of these structures is all that is needed for the establishment of conditioned reflexes; a function of the complexity and perfection of the conditioned reflex function of an organism must undoubtedly depend on the functioning of large complexes of nerve cells. This is the more probable inasmuch as the specified structures appear, to a greater or lesser degree, in all nerve cells, and not only in those of the cerebral cortex.

The methods at our disposal do not, as yet, permit of the demonstration of any structural peculiarities characteristic of the cortical cells, such as may be done in relation to such of their properties as is the specific closing function. This peculiarity of the functioning of cortical cells may perhaps be explained on the basis of the cytoarchitectonics of the cortex, i.e. on the way in which the cells are interconnected.

Thus, in speaking of the functional significance of the structures in question, we may say that their presence is an essential condition, although not, of course, the only one, for the origination of the closing function, and that in the absence of these structures this function cannot be accomplished.

Notwithstanding our reservations, we believe that our findings may possess some theoretical and practical interest, since they establish the significance of certain important intracellular structures for the accomplishment of the conditioned reflex function; in particular, they may assist in the elucidation of disturbances in cortical function encountered in various morbid conditions in which morphological investigations have shown changes in certain structural elements of cells. They may also be of assistance in determining the times of functional maturation of different parts of the cerebral cortex, using histological methods of investigation.

SUMMARY

The age at which conditioned reflexes are first able to be formed coincides in rabbits with presence of third stage (mature) neurons in all the layers of the cerebral cortex. The morphological criteria of maturity are: appearance of formed Nissl bodies, differentiation of the nucleoli, and appearance of synaptic knobs on the terminal dendrites.

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**Original Russian pagination. See C. B. Translation.

CHANGES IN THE METABOLITE CONTENT OF TISSUE PRESERVED BY V. P. FILATOV'S METHOD

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(Received January 15, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Tissue therapy is widely practised in hospitals in the Soviet Union and abroad. The practical application of the method of tissue therapy has to some degree determined its theoretical teratment. The mechanism of action of tissue transplants and the nature of the "biogenic stimulators" have not yet been satisfactorily explained. One way of shedding light on the chemical properties of the "materials of resistance" is by the study of the morphological and physiological changes in living tissues kept for various periods of time at a temperature of 2-4°C. Tissue preserved by Academician V. P. Filatov's method is, firstly, damaged tissue and secondly, is kept under conditions of partial asphyxia. It is known from reports in the literature that the nucleic acids, which play an important role in the vital processes of the body, are accumulated in tissue undergoing reversible damage [9, 10, 15, 17]. In conditions of asphyxia the glycogen content of the tissue diminishes and the lipid content increases [3, 6].

Our aim was to study the progress of the changes in the content of ribonucleic acid (RNA) in tissues during preservation. Furthermore, in several series of experiments a study was made of the changes in the glycogen and lipid contents of living tissues kept in conditions of hypothermia for various periods of time.

EXPERIMENTAL METHOD

The material used in the investigation was tissue from various organs (liver, tongue, intestine) from 28 guinea pigs and 8 rabbits, splenic tissue from cattle and human placenta. The material mainly used was liver from rabbits and guinea pigs. The tissues were preserved for various lengths of time (from one to ten days) by Academician V. P. Filatov's method, and after fixation they were examined for their content of RNA, glycogen and lipid.

In this investigation particular attention was paid to the study of the changes in the RNA content of the preserved tissue. According to data in the literature, one of the criteria of the RNA content is basophilia of the fixed tissue, which disappears during the action of the enzyme ribonuclease or of trichloracetic acid on the tissue [14]. Basophilia of the tissue, i.e. the ability of the fixed tissue to combine with a basic dye, was determined by Brachet's method in histological preparations and by photometry of alcoholic extracts of sections stained with a basic dye.

As several workers have shown [10, 11], changes in the combination of basic dye (diminution or increase of sorption of the dye) are found in association with changes in the content of basophilic substances (RNA) in the tissue. Arising from these investigations, in order to define the changes in the content of basophilic substances we also employed the method of photometry of alcoholic extracts of tissue sections stained with a basic dye. The results obtained by this method were compared with those obtained by Brachet's method.

In using the photometric method, we studied the basophilia of the tissue as follows: from tissue preserved for various periods of time at a low temperature, after fixation with 20% neutral formalin, sections were

prepared with a razor (in the case of the liver the area of the piece of tissue was 0.5 cm^2 and its weight from 45 to 55 mg). From 5-10 sections were taken from tissue for each day of preservation. Each section was stained with a 0.01% solution of neutral red, and decolorized with acidified alcohol. The amount of dye in the alcoholic extracts was judged by the extinction expressed in conventional units of a graduated photometer and calculated per 100 mg weight of tissue. Fixation of dye by unpreserved tissue, i.e. fixed immediately after decapitation of the animal, was conventionally taken as 100% (control). The changes in the fixation of dye by the tissue (decrease or increase) in the course of preservation were expressed as a percentage of the control.

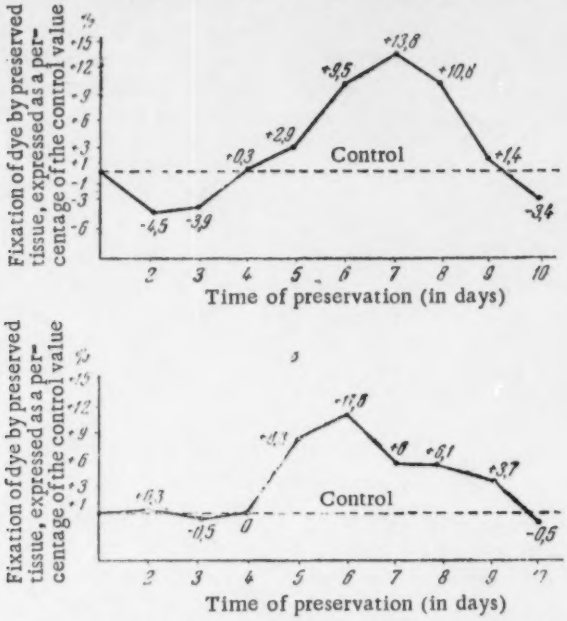

Fig. 1. Changes in the power of fixed tissue to combine with basic dye during the process of preservation of the tissue; a) liver tissue from guinea pigs (average of 23 experiments); b) liver tissue from rabbits (average of 8 experiments).

Glycogen was investigated in micropreparations obtained from sections of liver tissue from 5 rabbits by Best's method. Quantitative changes in the lipids were studied in preserved liver tissue from 5 rabbits and one guinea pig. The lipid was determined with Sudan III.

EXPERIMENTAL RESULTS

The experimental findings showed that in the process of preservation of liver tissue from rabbits and guinea pigs, the basophilia of the tissue as determined by the method of photometry undergoes the following changes (Fig. 1). The basophilia of the liver tissue, in the first 4 days of preservation, either remains the same as that of the unpreserved tissue, or even diminishes. Starting from the 5th day of preservation, the basophilia of the liver tissue of rabbits and guinea pigs increases and reaches a maximum for rabbit liver on the 6th day of preservation (11.6% ± 1.7 over the control value), and for guinea pigs liver — on the 7th day of preservation (13.8% ± 2.8 over the control value). After the 8th day the sorption of basic dye by the tissue begins to fall.

The same changes in the basophilia of the tissue in the process of preservation were clearly demonstrated by Brachet's method on micropreparations as by the method of photometry. In the first days of preservation, for instance (2nd-4th day) the basophilia of the liver tissue is no greater than that of control sections. On subsequent days (6th-8th day) a sharp increase in the intensity of staining of the sections of liver tissue is observed, after which the pyroninophilia begins to show a marked fall. Thus the determination of the basophilia of the tissue after various periods of preservation by the different methods gave compatible results.

The changes in the basophilia of the tissue in the course of its preservation are evidence of changes in its content of RNA. The progress of the changes in the RNA content of liver tissue of rabbits and guinea pigs, which we established, enabled us to subdivide the whole period of preservation into 3 periods. In the first period of preservation (from the 1st to the 4th day) the RNA content of the tissues shows no increase in comparison with that of unpreserved tissue, and the RNA content of liver tissue from guinea pigs even diminishes. In the second period of preservation (from the 5th to the 8th day) the RNA content of the tissues increases successively from day to day, reaching a well marked maximum on the 6th-7th day of preservation. Tissue in the second period of preservation, i.e. preserved for that length of time advised by V. P. Filatov for transplantation to patients, is characterized according to data in the literature [2, 4, 11, 16] by physiological processes which proceed with considerable energy. In the third period of preservation (after the 9th day) the RNA content of the tissue, according to our findings, falls sharply. It has been shown by work emanating from V. P. Filatov's school [2, 12] that profound autolytic processes develop in tissue which has been preserved for 9 days and more.

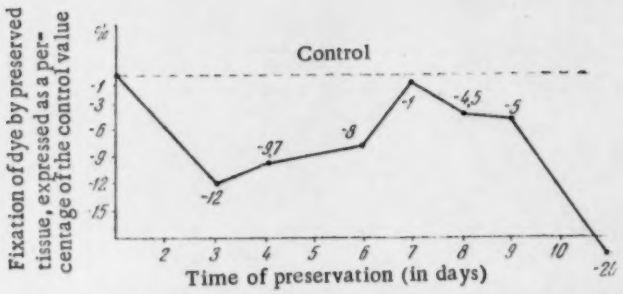

Fig. 2. Changes (in per cent) in the power of combination with basic dye (in the course of preservation) of fixed liver tissue from guinea pigs with avitaminosis in the process of preservation of the tissue. Mean results of 5 experiments.

In addition to studying the changes in the basophilia of the liver tissue in the course of preservation, we examined the changes in the basophilia of the tissue of other organs (spleen of cattle, human placenta, tongue and intestine from 7 guinea pigs and 6 rabbits). The basophilia of the tissue of these organs also increases during our second period of preservation (on the average from 10 to 33%). Growth of basophilic substances (RNA) in the preserved tissues may evidently take place by various methods. First of all its is admitted that there is an increase in the content of free RNA as a result of its liberation from its bond with protein. It is possible that an increase in the quantity of polynucleotides or of acid groups unconnected with protein may bring about intensification of the basophilia of the preserved tissue; it is also possible that rupture of the bond between neighboring nucleotides within the same molecule of nucleic acid may lead to an increase in the sorption of basic dyes by the tissue. The experimental results of S. M. Shibaeva [13] show that a process of depolymerization of nucleic acids takes place in preserved tissue. The fall in the basophilia of the tissue in the third period of preservation is due, in our opinion, to the departure of basophilic substances from the tissue. Flushing of RNA (basophilic substances) from tissue suffering damage has been observed by many workers [1, 7-9].

Our experimental findings from the study of the changes in the lipid and glycogen contents of preserved liver tissue from rabbits and guinea pigs showed clearly that from the first days of preservation the glycogen content of the tissue diminished sharply, whereas the lipid content rose. Our observation that there is a fall in the glycogen content and a rise in the lipid content confirms the findings obtained previously by V. P. Filatov's school [5].

We thus established that in tissue undergoing preservation quantitative changes take place in the contents of RNA, glycogen and fat. At strictly determinate periods of preservation there are regular changes in metabolism, evidently connected with the differing intensity of damage to the tissue. The changes in the metabolism on the 6th-7th-8th day of preservation are of the utmost interest, since it is tissue at this stage of preservation which is recommended by V. P. Filatov for transplantation. Our findings show that there is an increase in the RNA content of tissue at this period of preservation.

V. P. Filatov and his school consider that "materials of resistance" accumulate in preserved tissues taken from young and healthy animals; for this reason we studied the character of the changes in the RNA content of preserved liver tissue taken from healthy guinea pigs and from guinea pigs with avitaminosis. In full agreement with V. P. Filatov's views on the accumulation of biostimulators in preserved tissues taken from healthy animals, according to our findings during preservation of tissue an accumulation of RNA on the 5th-8th day takes place also, but only if the tissue is taken from healthy animals. The basophilia of liver tissue taken from 5 guinea pigs with avitaminosis did not exceed that of the control on any one day of preservation or in any single experiment (Fig. 2).

According to V. P. Filatov's concept of tissue therapy, the tissue of animals kept in arduous conditions of existence and without preservation, contains a large quantity of biostimulators. According to our findings, unpreserved liver tissue from 5 guinea pigs with avitaminosis contained significantly more RNA (on the average 20% more) than did the unpreserved liver tissue from healthy animals. Considering in the first place the important role of RNA in the vital processes, and in the second place the parallel changes in the therapeutic activity and the RNA content of preserved tissue, it may be postulated that RNA is a component part of the biostimulators. It is also admitted that the increase in the RNA content of preserved tissue may be used as a test of the preparedness of the tissue for transplantation to patients.

SUMMARY

The author studied the dynamics of the changes occuring in the content of basophilic substances (RNA) glycogen and fats in the tissues preserved by the method of academician V. P. Filatov. The total period of preservation may be subdivided into 3 stages. During the 1st stage of preservation (from the 1st to the 4th day) the quantity of the basophilic substances remains unchanged or decreased as compared to the control. The quantity of RNA is greatly increased from the 5th to the 8th day (the second stage), while after the 8th day the amount of RNA shows a rapid reduction. If the tissue is taken from sick animals basophilia does not increase during the process of preservation. The quantity of glycogen is sharply diminished from the very first days of preservation, while the quantity of fat rises.

By estimating the increased quantity of the basophilic substances in the preserved tissue one may judge the readiness of the tissue for transplantation to the patients.

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THE INFLUENCE OF THE MITOTIC REGULATORS ON THE NUCLEIC ACID CONTENT OF THE CELL

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The processes of cell division in the animal body are under the control of a neurohumoral regulatory system. In this regulation an important part belongs to the hormone of the adrenal gland. Adrenalin is a powerful inhibitor of cell division and has a wide range of activity [1-5, 11]. The adrenal cortical hormone – cortisone – also possesses antimitotic activity, but its influence on cell division is more selective. Cortisone reduces the mitotic acitivity in the lymphoid tissues and, to a lesser degree, in the ectodermal epithelium. It has no action on the division of the epithelial cells of the intestine [1, 3]. The mode of action of the mitotic regulators on cell division is not yet clear. It has been shown by several histochemical [9, 10 and others]* and experimental [6, 7] investigations that one of the fundamental biochemical processes with which cell division is connected is the metabolism of the nucleoproteins. These findings suggest that the influence of the mitotic regulators on cell division, and in particular that of adrenalin and cortisone, is effected through the nuclear metabolism of the cell.

In this connection we undertook experiments in which histochemical methods were used to study the nucleic acid content of cells during inhibition of mitosis by adrenalin and cortisone.

EXPERIMENTAL METHOD

Experiments were carried out on white mice of the same litter, 2 months old. Investigations were made of the epithelium of the cornea, skin, tongue and intestine and of the lymphocytes of the thymus gland and the lymphatic glands. In addition to these cells, which possess high mitotic activity, studies were made also of the liver cells, which in adult animals lose their power of mitotic division. These cells were selected mainly on account of their accessibility for histochemical investigation. The material was fixed in Gall's mixture. Ribonucleic acid (RNA) was revealed by pyronine and methyl green by Brachet's method, and desoxyribonucleic acid (DNA) by the Feulgen reaction.

In the first series of experiments the nucleic acid content was studied after injection of the animals subcutaneously with adrenalin in doses of 0.05, 0.5 and 1 mg/kg body weight of the animal. Injection of the drug was repeated twice at intervals of $1\frac{1}{2}$ hours. The animals were killed 1 hour 30 minutes, 3 hours and 4 hours after the last injection. Selection of the dosage of the drug and the time of examination of the material was dictated by findings obtained in the laboratory on the changes in mitotic activity during similar experiments. As a control to this series of experiments white mice were used, kept under identical conditions but not receiving adrenalin.

In a second series of experiments white mice were injected subcutaneously or intraperitoneally with cortisone in a dose of 2.5 mg/kg body weight. In the first group of these experiments cortisone was injected once, in the second group – twice in the course of 24 hours, and in the third group 5 injections of cortisone were given in the course of 72 hours. In all the groups of experiments the material was examined 4 hours after the last injection. For a control animals were used which were kept under identical conditions and received injections of corresponding doses of physiological saline. The number of injections and the time of killing of the animals were determined from consideration of the findings on changes in mitotic activity in similar experiments.

*As in original

EXPERIMENTAL RESULTS

The results of the first series of experiments showed that the injection of adrenalin not only caused a fall in the mitotic activity but considerably altered the nucleic acid content of the cell. Injection of adrenalin in a dose of 0.05 mg/kg body weight causes a marked fall in the DNA and RNA contents of the corneal epithelium and the liver cells. In the epithelium of the skin, the tongue and the intestine no changes in the nucleic acid content were observed. Analagous results were given by the injection of adrenalin in a dose of 0.5 mg/kg body weight. The clearest results were obtained after injection of adrenalin in a dose of 1 mg/kg body weight. In this case an obvious fall in the RNA and DNA contents was observed in the epithelium of the cornea, the skin and the tongue and in the liver cells (Fig. 1). In the intestinal epithelium and the lymphocytes of the thynus and lymphatic glands, no fall in the RNA and DNA content was observed even after a second injection of adrenalin. Only in isolated experiments in which small quantities of adrenalin were injected was there a slight fall in the nucleic acid content of the lymphocytes.

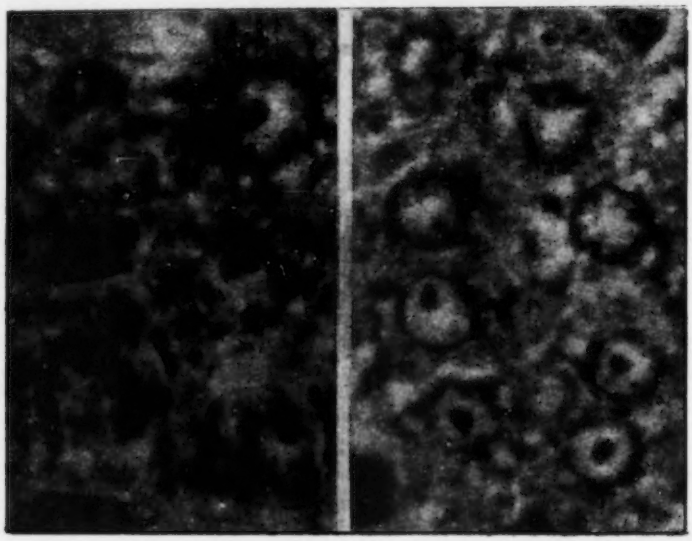

Fig. 1. The nucleic acid content of the liver cells. On the left – control; on the right – after injection of adrenalin.

The results of this series of experiments thus show that the injection of adrenalin in dose causing a fall in mitotic activity cause a fall in the RNA and DNA contents of the cells also.

The reaction of cells of different tissues to the injection of adrenalin is not the same. The sharpest changes are observed in the corneal epithelium and the liver cells; less marked changes in the nucleic acid content are found in the epithelium of the skin and tongue, and in the intestinal epithelium and the lymphocytes of the thymus and lymphatic glands changes are either absent or inconstant and feebly expressed. Comparison of these findings with those on the effect of adrenalin on mitotic activity indicates a quite clear parallel between the changes in the nucleic acid content of the cells and the mitotic activity. Injection of adrenalin causes an obvious antimitotic effect in the epithelium of the skin, the tongue and, in particular, the epithelium of the cornea. In the lymphoid organs adrenalin does not alter the mitotic activity. In the intestinal epithelium the mitotic activity is changed only relatively slightly by the action of adrenalin, and changes are not seen in all the experiments.

In the second series of experiments we investigated the influence of cortisone on the nucleic acid content of the cells. A single injection of cortisone caused an obvious fall in the RNA and DNA contents of the lymphocytes and the thymus and lymphatic glands and in the liver cells. There was an insignificant fall in the nucleic acid content of the epithelium of the cornea and skin. No changes were observed in the RNA and

DNA content of the cells of the intestinal epithelium. Similar findings were obtained after two injections of cortisone. A very severe fall in the nucleic acid content of the lymphocytes of the thymus and lymphatic glands and of the liver cells was observed after repeated injections of the drug. The basophilic clumps and rods, characteristic of the normal cells, disappeared from the cytoplasm of the liver. Only a small perinuclear area of the cell and a narrow border of cytoplasm adjacent to its membrane gave a reaction for RNA (Fig. 2). In the intestinal epithelium there was no change in the RNA and DNA contents of the cells even after repeated injections of cortisone.

The results of the experiments with cortisone thus show that the injection of this drug causes a fall in the nucleic acid content of the cells.

In both the adrenalin and cortisone experiments a quite clear parallel is observed between the mitotic activity and the changes in nuclear metabolism. Cortisone suppresses mitotic activity mainly in the lymphoid organs. Cortisone does not alter the mitotic activity in the intestinal epithelium [1, 3]. The liver occupies a peculiar position, for in adult animals no mitoses are present there.

In regeneration, however, when proliferation of liver cells takes place, cortisone then has a quite considerable antimitotic effect [15].*

In experiments in which cortisone was injected, besides studying the changes in the nucleic acid content, we measured the size of the liver cells. These measurements, carried out by A. N. Chizhova, showed that under the influence of cortisone a marked diminution takes place in the dimensions of the cells and their nuclei, and there is an obvious shift of the variational curves to the left. These measurements suggest that under the influence of cortisone there is not only a relative fall in the concentration of RNA and DNA, as some authors suggest [8], but a true fall in the nucleic acid content of the cell. Thus in spite of the inconsistency of the results of the biochemical investigations dealing with the effect of cortisone on nuclear metabolism [12-14] and others], we may conclude that cortisone causes interference with the synthesis of RNA and DNA in the cell.

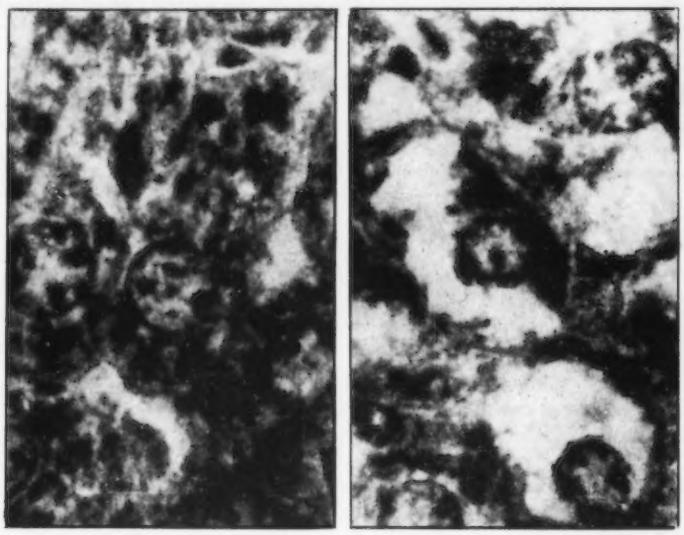

Fig. 2. Nucleic acids in liver cells. On the left – control; on the right – after injection of cortisone.

The results of the experiments performed show that both adrenal hormones, possessing a powerful antimitotic action, reduce the RNA and DNA contents of cells. Considering the role of nuclear metabolism in the process of mitotic division, it may be supposed that the antimitotic action of adrenalin and cortisone is connected with interference with the synthesis of nucleic acids. Naturally the influence of the adrenal hormones on nuclear metabolism may be effected not directly, but through other intermediate metabolic links. In one way or another the results of our experiments suggest that adrenalin and cortisone influence cell division

*As in original.

ultimately through nucleoprotein metabolism. Later experiments will show whether this mechanism extends to other neurohumoral factors concerned in the regulation of cell division in the animal body.

SUMMARY

Administration of adrenalin reduces the mitotic activity and considerably changes the content of nucleic acids in the cells. Adrenalin decreases the content of RNA and DNA in the cornea epithelium, hepatic cells and, to a lesser degree in the skin and tongue epithelium. Cortisone reduces the quantity of nucleic acids in the lymphocytes of the thymus gland, in the lymph nodes and hepatic cells. The antimitotic effect of the adrenal gland hormones is evidently connected with the disturbed cellular synthesis of the RNA and DNA.

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AN EXPERIMENTAL HISTOLOGICAL INVESTIGATION OF THE EPITHELIUM OF THE CERVICAL CANAL OF THE UTERUS IN HUMANS

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The epithelium of the cervical canal of the human uterus and its glands are derived from the Müllerian ducts. Although distinguished by great uniformity in physiological condtions, the epithelium of the cervical canal shows a wide range of variation in pathological processes in humans (proliferation [6], pregnancy and hormonal changes [9, 16, 17, 18], and tumors [3, 4, 21]) and also in animals under experimental conditions [5, 10, 11]. In this situation in particular may be formed structures composed of many rows or layers whose origin and nature are the subjects of animated discussion at the present time.

This paper is devoted to a study of the changes in the structures of the cervical epithelium of the human uterus by the explantation method.

EXPERIMENTAL METHOD

As material for implantation we used particles of the mucous membrane of the cervical canal, obtained from women undergoing operation for various diseases of the body of the uterus. The patients were aged from 28 to 48 years. Altogether 14 series of experiments were carried out. Cultivation was done in flasks. The nutrient medium used was plasma and embryonic extract of chicks, human group AB serum and Tyrode solution. To the nutrient medium was added a mixture of penicillin and streptomycin in a dose of 0.1 μg per flask. The maximum period of cultivation was 35 days. Altogether 225 cultures were studied, living and in fixed preparations, in both total and serial sections. The fixing agents were 15% formalin and Zenker – formol. Stains: Carazzi's hematoxylin, Heidenhain's iron – hematoxylin, mucicarmine, hematoxylin – eosin, azure P – eosin, van Gieson or Mallory. In order to demonstrate the cell margins the preparations were treated with silver nitrate.

EXPERIMENTAL RESULTS

Cultivation of the cervical epithelium is associated with great difficulty on account of the pronounced fibrinolysis. The inoculated fragments as a rule floated freely after only 24 hours within a cavity of liquefaction. Repeated daily passage of the floating fragments into new flasks was required before it was possible to obtain extensive growth of epithelium. This is a characteristic property of explantates of epithelia derived from the Müllerian ducts [1, 2, 7, 8]. Fibrinolysis was more pronounced the greater the formation of mucus. It falls considerably on the 5th-10th day of cultivation. Extensive growth in the form of membranes is observed relatively rarely, and usually epithelialization of the central fragment occurs. Growth in the cultures is brought about by the epithelium lining the canal and the epithelium of the glands. Only a few hours after inoculation characteristic outgrowths of epithelium appear at the margin of the explantate in the form of folds and of rounded, spherical or elongated formations, freely projecting into the surrounding nutrient medium. Initially these outgrowths have a compact structure. Later, as a result of disintegration of their central part

Fig. 1. Integumentary epithelial cell layers. a) Cells of differing height in the composition of the layer; celloidin section, 15 day culture, Zenker formol, hematoxylin – eosin; b) single-layered high prismatic integumentary epithelium, dome-shaped apical surfaces of the cells; celloidin section, 15 day culture, Zenker formol, hematoxylin – mucicarmine; c) multi-layered epithelializing cell lamina, cells cylindrical in shape with dome-shaped apical surfaces bound a cavity; celloidin section, 12-day culture, Zenker – formol, iron – hematoxylin. Magnification 400 x.

and of a general increase in their dimensions, they are converted into vesicles and tubes with a single-layered epithelial wall.

The cervical epithelium rapidly epithelializes the surface of the explantate. Complete epithelialization takes place on the 2nd - 3rd day. The integumentary layer, as a rule, has a single-layered and single column structure. Differentiation begins relatively early in this layer and takes place not simultaneously throughout the layer, but in the form of islets. As a result of this the fragment appears to be covered with epithelium in which there is alternation of areas consisting of condensed isoprismatic and high prismatic cells

(Fig. 1, a). Sometimes the whole covering epithelium of the explantate consists of squamous or cubical cells among which are found only small groups of a few high prismatic cells.

Fig. 2. Growth of connective tissue within a fragment, mitosis in single-layered integumentary epithelium, celloidin section, 16 day culture, Zenker formol, hematoxylin – eosin. Magnification 600 x.

The cells and nuclei of the integumentary epithelium in early stages of cultivation are distinguished by the diversity of their shapes and sizes. With increasing age of the cultures the integumentary epithelium acquires a more uniform and differentiated character, often with well marked interlocking zones. In some cases the explantates are entirely covered by a single layer of high prismatic cells, which lie in close contact with each other and have a convex, dome-shaped free surface (Fig. 1, b) and one or several oval nuclei situated at the base. In the epithelialized areas a large mitoses is seen, distributed apically. The integumentary epithelium retains this character until the terminal periods of cultivation. Similar structures in the integumentary epithelium are observed in cultures from the mucous membrane of the uterus [2] and oviducts [1] of the rabbit, and in cultures from endometriosis [7], the epithelium of the tubes and certain ovarian cysts in humans [8].

In isolated cases, under the layer of high prismatic cells in the integumentary epithelial layers are found cells which are round in shape and with a lightly stained nucleus, containing fine chromatin granules. In our material we were unable to see whether these cells were arranged in groups or in a continuous layer. They were usually dotted in one by one. It is difficult to say whether they correspond to the "subepithelial" or "reserve" cells which are described in the cervical epithelium of the uterus [6, 19, 20], although such an impression seems probable.

In the period of most acitve proliferation of the epithelium areas consisting of many columns or layers are formed in addition to the single-layered integumentary cells. The number of layers here may reach 5 or more. These areas are distinguished from the multi-layered integumentary cells in cultures of epidermal epithelia by the absence of vertical anisomorphism in all stages of cultivation, and consist of solitary round or slightly flattened cells. Mitoses are distributed at all levels of the multi-layered cells. The cells of the superficial layer are often prismatic in shape, with a characteristically convex apical surface and a nucleus situated basally. Sometimes these cells bound a cavity formed as a result of mucin formation and degeneration of cells (Fig. 1, a). Over the whole surface of the multi-layered area there lies a thin film of mucus. The characteristic form of the cells and the presence of mucous secretion indicates that these multilayered structures are derivatives of the cervical epithelium of the uterus.

The multilayered structures formed during cultivation of epithelium from the cervical canal cannot be regarded as an accidental formation or as the result of accumulation of cells [1, 2, 15].

There is no doubt that the epithelia developing from the Müllerian ducts can under certain conditions (chemical irritation, avitaminosis B, hormonal changes, pathological proliferation, explantation) form multilayered systems of cells, which are not transitional structures but which are preserved in explantates for 30 days or more. In previous work devoted to the celonephrodermal group of tissues [1, 2, 7, 8, 12, 13, 15], insufficient attention was directed to these structures and they were not correctly interpreted. There is now no doubt that they must be regarded as a manifestation of specific histoblastic forerunners of epithelia, derived from the Müllerian ducts. However, these multilayered structures, as may be seen from a comparison of our findings in work on the cervical and vaginal epithelium, are by no means identical with each other, and during explantation they firmly retain their qualitative differences. The multilayered structures of the cervical epithelium do not differentiate according to the epidermal type and in contrast to the vaginal epithelium they form mucus.

During full epithelialization of the central fragment with a single layer of cells the growth of connective tissue cells inside the explantate does not cease, as takes place in explantates which are epithelialized by epidermal cell systems. On the contrary its cells proliferate and multiply by mitosis. The connective tissue acquires a "cytogenic" character and stays viable for 4-5 weeks (see Fig. 2). Presumably the epithelium of the cervical canal in contrast to epithelium of the epidermal type, and in particular vaginal, does not prevent to any appreciable extent the interchange of materials between the central part of the explantate and the nutrient medium.

Extensive growth of the epithelium in the form of membranes is seen less often than epithelialization and it begins considerably later, when fibrinolysis has become weaker. Very slender, always single-layered and extensively growing membranes are easily crumbled and soon die. For this reason they rarely attain a considerable size. Membranes of cervical epithelium differ sharply in the shape and arrangement of their cells from membranes of all other human epithelia and from the epithelia of the uterus and oviducts in the rabbit [8]. In them can be clearly distinguished alternating and dissimilar areas of varying extent, consisting of elongated dark and wide, longitudinally splitting lightly staining cells. The mutual arrangement of the cells, their arrangement and distribution in the different planes create a peculiar wave-like pattern which is accentuated by the uneven staining of the cytoplasm (Fig. 3, a). Membranes of this pattern are encountered only in cultures of epithelia derived from the Müllerian ducts in humans, and are regarded as an expression of species specificity. V. P. Mikhailov [8] calls this peculiar pattern of these membranes in explantates of the epithelium of the Fallopian tube and of certain ovarian cysts "watered silk" and considers that this sign may be used as a decisive proof of the origin of a cultivated tissue from the Müllerian ducts.

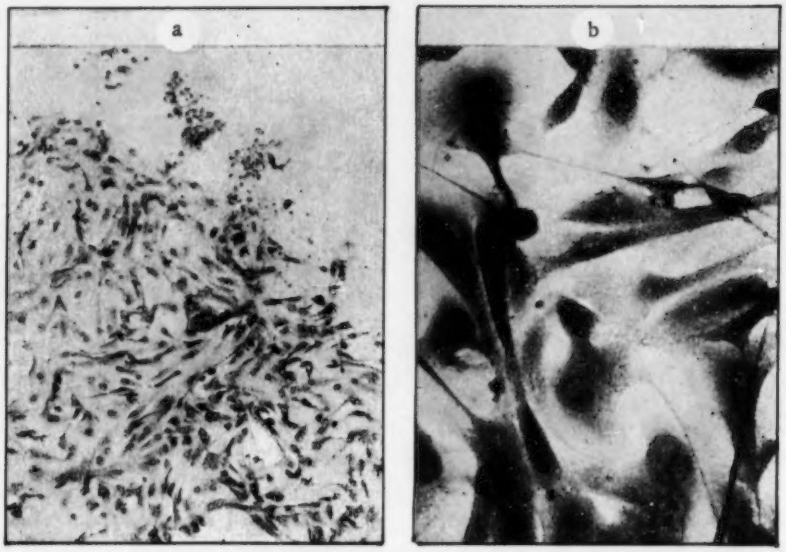

Fig. 3. Epithelial membranes. a) Usual appearance, peculiar wavelike pattern of the membrane, total preparation, 13 day culture, formalin, Carazzi hematoxylin, magnification 80 ×; b) detail of the membrane with a wave-like pattern, elongated and longitudinally splitting cells, long narrow cytoplasmic processes situated in different planes; total preparation, 15 day culture, dormalin, Carazzi hematoxylin, magnification 400 ×.

The cells in the membranes are characterized by marked polymorphism and by considerable variations in their size. The perinuclear part of the cytoplasm stains very intensely but towards the periphery the staining becomes less intense and the cell margin is hardly distinguishable. For this reason the wrong impression may be created that the cells do not lie next to each other but are separated by intervals. The cell margins are not clear in sections stained with hematoxylin and they are well shown only when stained with silver.

The cells are often equipped with long, fine cytoplasmic processes which connect cells far removed from each other (Fig. 3, b). The processes merge with the substance of the membranes, cross each other, are distributed in different planes and form a complex network on the free surface of the membrane. The round nuclei usually contain 1-2 nucleoli and fine chromatin granules. The nuclei are situated in the center of the cell, eccentrically or even in one of its processes.

For this reason their distribution is often highly uneven. The majority of the cells are mononuclear, but occasionally cells with 2-4 nuclei are seen.

Particularly in the early stages of cultivation numerous mitoses are found in the membranes, up to 8-10 in a field of vision of a 40 × objective. Amitoses and fragmentation of nuclei are seen relatively rarely. They are commoner in aging cultures. Rare discoveries, but worthy of attention, include solitary cells with ciliae in the composition of some membranes. In living preparations the waving motion of these living ciliae was observed.

Only in isolated explantates is an extensive mixed growth of epithelium and connective tissue observed. This takes place only in cases where the connective tissues, quantitatively predominant, is able to grow into the fibrin before it begins to liquefy and the fragment epithelializes.

Throughout all periods of cultivation secretion of mucus is observed in explantates of cervical epithelium In the first few days after inoculation an abundant formation of mucus takes place both by the cells of the living epithelium and by the cervical glands which happen to be present in the fragment. Glands distended with mucus may open out on the surface of the explantate and their contents be liberated. Mucus formation gradually diminishes. Mucus is formed as tiny inclusions and small vacuoles in the cells of the membranes and of the integumentary epithelial layers, both single- and multilayered, and also as a narrow film on their surfaces. In the high prismatic cells the whole of the cytoplasm above the nucleus stains intensely with mucicarmine. In contrast to this there are indications [1] that in a particular case active formation of mucus may take place by the growing cells themselves and not by the preservation of inclusions of secretion already present in the explanted material.

Thus the epithelium of the human cervical canal in culture not only shows certain histological properties typical of and common to tissues of celonephrodermal type [14], but also reveals profoundly distinctive features and species specificity. The epithelium of the cervical canal, normally uniform, shows considerable plasticity in cultural conditions, giving rise to high prismatic cervical epithelium and to undifferentiated uterine epithelium, and forming structures characterized by many layers and columns. The formation of mulitlayered structures by the cervical epithelium is connected with active proliferation of its cells.

Summing up its histological typical properties as revealed during explanation, the epithelium of the cervical canal may be sharply and firmly distinguished from the epidermal epithelia, including the epithelium of the vagina which we have ourselves studied. This is shown by a difference in the fibrinolytic activity, by the character and the time of growth, the number and situation of mitoses, the secretory function, the structure of membranes and epithelializing layers, by the different trends of their differentiation and by their cytological peculiarities.

SUMMARY

Culturing of normal epithelium of the cervical canal is associated with pronounced fibrinolysis and mucus secretion.

Single-layered, multirowed and multilayered strata are formed in epithelization of various sections. The latter differ from the multi-layer strata of the epidermal type by the absence of the vertical anisomorphicity and by the presence of mucus secretion. They should be considered as the manifestation of the specific histoblastic potentiality of epithelia, derived from the Müllerian ducts.

Peculiar membranes with characteristic wave-like "moire" pattern are formed in extensive growth, which is the manifestation of the species-specificity of the human Müller's epithelium. The cervical canal epithelium shows a great difference from the epithelia of the epidermal type.

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MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN THE SMALL INTESTINE OF THE DOG AFTER ITS PARTIAL RESECTION

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In experimental investigations dealing with resection of the intestine it has been discovered to what extent the intestine may be resected without any material harm being done to the animal [2, 8], how the assimilation of the principal food substances proceeds after this proceudre [1, 2, 4] and how the movements of the residual portion of the intestine are altered [5]. V. P. Mandzhgaladze[3], studying the enzyme-secreting function of the intestine, found that after resection of the proximal or distal divisions of the small intestine there is an increase in the concentration of enzymes per 1 g of solids in the intestinal juice secreted by the isolated portion. The percentage ratio of the solid part of the juice is diminished after resection of the proximal division, and increased after resection of the distal division. In these experiments the isolated area corresponded to the section of intestine removed and not to the residual portion. Nevertheless it would be of the utmost interest to trace the restoration of the function of the residual portion of the small intestine and the isolated area corresponding to it.

The question of the time of development of the compensatory reaction arising after removal of a portion of the intestine has not been discussed, and in connection with the character of the morphological changes taking place under these conditions the findings are contradictory.

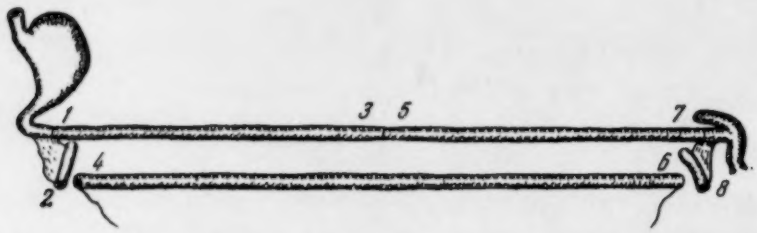
In view of the interdependence of structure and function in the intestine we undertook a morphological and physiological investigation of the changes arising in the small intestine after partial resection of that organ.

EXPERIMENTAL METHOD

Two areas of the intestine were isolated in 5 dogs by Thiry's method: one immediately after an area of the intestine with a short mesentery, the other at a distance of 10 cm from the ileocaecal angle. The periodic secretion of intestinal juice in the course of 4 hours was studied (18 hours after taking food). The volume of juice and the proportions of solid and liquid parts were noted. The juice was homogenized and its enterokinase content was determined by Shlygin's method [7] and its alkaline phosphatase content by the method of Fomina, Mikhlin and Shlygin [6]. After establishing the basic level of the intestinal secretion (in 8-10 experiments on each dog), and after starvation for 24 hours, from $^1/_3$ to $^1/_2$ of the middle portion of the small intestine of the dogs was resected. On the 2nd-3rd day after operation, and subsequently every 1-3 days, the dogs were mounted on the apparatus. In addition, half the small intestine, from the middle portion, was resected in 6 dogs without the preliminary formation of isolated areas.

At different times from 2 weeks to 6 months after operation the dogs were killed by means of an electric current. Before slaughter the dogs were starved for 24 hours. During the operation and after slaughter pieces of intestine were removed from the dogs for histological examination in accordance with the following scheme (see Figure).

In order to investigate the enzymes a 1:5 aqueous extract was prepared from scrapings of the mucous membrane (time of extraction – 30 minutes), and its enterokinase and phosphatase contents determined. For the histological examinations the material was fixed in 12% formalin solution and then embedded in paraffin wax. Sections were cut to a thickness of 10 μ and stained with hematoxylin – eosin.



Scheme of the operation – resection of the intestine and removal of samples for histological examination. 1) Beginning of the jejunum; 2) anterior isolated area; 3) midpoint of the jejunum; 4-6) resected area; 5) midpoint of the ileum; 7) end of the ileum; 8) posterior isolated area. Areas 1 and 2, 3 and 4, 5 and 6, 7 and 8 were compared.

EXPERIMENTAL RESULTS

The periodic secretion of intestinal juice and its enzymic activity. The volume of juice secreted from the anterior isolated area was only slightly changed after the operation, as shown by Table 1 in which the mean values for each dog taking part in the experiment are given.

The volume of juice secreted from the posterior isolated area of dogs Nos. 1 and 4 fell after a prolonged postoperative period (2-6 months); this may be connected with the fact that this particular area begins to be epithelialized from the surrounding skin and is gradually overgrown. In the same dogs, as time went on the proportion of solid matter in the juice rose (Table 2). The increase in secretion in dog No. 5 can be accounted for by prolapse of the mucous membrane of this isolated area. Where the isolated area of intestine was in good condition (as in dogs Nos. 2 and 3) the secretion was not appreciably changed.

TABLE 1

Mean Volume of Intestinal Juice (in grams) During 4 Hours' Secretion

Dog No.	Area of intestine			
	anterior		posterior	
	before operation	after operation	before operation	after operation
1	5,28	4,60	3,52	2,28
2	2,78	4,83	2,0	1,53
3	6,81	5,68	4,47	4,56
4	3,06	3,98	2,03	0,62
5	3,45	2,98	0,87	2,01
Average for all dogs	4,37	4,41	3,33	2,79

The proportions of the solid and liquid parts of the juice were essentially unchanged after the resection (Table 2).

The findings in connection with the enterokinase content of 1 g of the solid part of the intestinal juice secreted from the anterior isolated area before and after resection are given in Table 3.

TABLE 2

Mean Percentage Value of the Solid Component of the Intestinal Secretion Before and After Resection of the Intestine

Dog No.	Area of intestine			
	anterior		posterior	
	before operation	after operation	before operation	after operation
1	30,9	37,5	30,0	28,4
2	45,6	26,5	28,0	50,3
3	32,9	40,0	41,4	35,7
4	49,5	43,7	35,4	79,1
5	43,4	61,3	59,1	47,5
Average for all dogs	40,4	41,8	38,7	40,9

TABLE 3

Mean Number of Units of Enterokinase in 1 g of Solids in the Intestinal Juice

Dog No.	Before operation	After operation
1	774	710
2	863	984
3	447	533
4	470	521
5	726	509
Average value for all the dogs	656	651

TABLE 4

Average Number of Units of Alkaline Phosphatase in 1 g of Solids in the Intestinal Juice

Dog No.	Area of intestine			
	anterior		posterior	
	before operation	after operation	before operation	after operation
1	20 000	49 300	11 400	9 200
2	70 100	96 400	10 600	20 900
3	73 200	525 700	19 700	123 500
4	63 500	386 600	9 100	70 000
5	477 300	757 000	37 000	66 500
Average for all dogs	140 900	363 000	17 600	58 000

As seen in Table 3, the enterokinase content varied between approximately the same limits before and after resection. The volume of juice and the proportion of solids were not appreciably altered, and so the number of units of enterokinase during secretion for 1 hour remained the same after operation as it had been before.

There was a change in the phosphatase content in the form of an increase in its concentration per gram of solid matter and per hour of secretion in both the anterior and posterior isolated areas. The mean values for each dog taking part in the experiment are given in Table 4.

We observed an increase phosphatase content of the intestinal juice even in the first few days after resection, i.e. on the 2nd-3rd day, when for the first time after the operation the dog was mounted on the apparatus. Later on the phosphatase content fluctuated but did not fall below the upper limits of normal.

The enzymic activity of the mucous membrane. The enterokinase concentration in the mucous membrane of the small intestine began to rise in the 2nd-3rd weeks after resection of the intestine, reaching a maximum after 1-2½ months (a two- or threefold increase). The increased enzymic activity was maintained for 6 months after operation. In respect of the phosphatase activity of the mucous membrane no definite regular relationships could be demonstrated.

Morphological modifications in the intestinal wall. At necropsy of the experimental animals roughly one month after operation some increase in the diameter of the intestine and some thickening of its wall could be seen. Kerkring's folds were more pronounced than during the resection. This was especially noticeable in dogs in which the postoperative period was from 2 to 4 months.

On microscopic examination we discovered hypertrophy of the mucous membrane. Measurements showed that the mucosa of the portion of intestine remaining after resection (beginning 1 month after operation) was from 20-30% thicker than the mucosa of the resected area of intestine of the same dog. This thickening was due to a proportionate lengthening of the villi and crypts. Kerkring's folds also became much thicker and longer after operation. The number of cells in the same extent of the epithelial layer of the villi in the resected part differs hardly at all from their number in the remaining portion of the intestine at whatever time the animals were killed. From this it may be concluded that the process of hypertrophy of the mucous membrane takes place on account of hyperplasia of its cellular elements.

Hypertrophy is noted in some dogs only 2-3 weeks after the operation, reaching a maximum after a period of 2 months, and it is observed in both the jejunum and ileum.

The processes of morphological modification almost pass by the isolated areas; the thickness of the mucosa of the isolated areas was from 20-40% less than the thickness of the mucosa of that section of the intestine from which the area had been isolated.

On the basis of our experiments we cannot conclusively decide whether an increase takes place in the total number of villi. This can only be judged after a study of intestine which has been fixed in an atonic state.

In comparing the findings on the morphological and enzymic changes, attention is drawn to the fact that the degree of morphological modification does not always correspond to the degree of stimulation of enzymic activity. Evidently the processes of functional compensation and of morphological modification supplement each other.

The mucosa of the isolated areas reacts differently to resection of the intestine from the mucosa of the residual portion of the intestine.

It may be postulated that the restorative processes in the intestine after resection are directly influenced by the passage of food masses.

It may be concluded from the results obtained that after resection of the middle part of the intestine both the jejunum and the ileum take part in the restorative processes.

SUMMARY

About $^1/_3$ to $^1/_2$ of the middle part of the small intestine was resected in dogs. Secretion of the juice by the isolated portions of jejunum and iluem, as well as the content of enterokinase do not change after the resection. The activity of basic phosphatase in 1 gm of the solid part of intestinal juice per hour of secretion increases by 2-3 times after the resection. The content of enterokinase in the scrapings of the mucous membrane of residual part of intestine increases by 2-3 times in a month after the operation. By this time the thickness of the mucous membrane is increased by 20-30%. This is not observed in the mucous membranes of the isolated intestinal portions. Morphological change and increase of the fermentative activity were noted in the mucous membrane of the jejunum and ileum.

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THE ROLE OF THE NERVOUS SYSTEM IN THE PROCESSES OF TISSUE REGENERATION

REPORT III. STIMULATION OF THE CEREBRAL CORTEX AND THE HEALING OF WOUNDS OF THE SKIN

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In our previous reports [6-7] it was shown that disturbance of peripheral innervation either by section of the great auricular nerve or by extirpation of the cervical sympathetic ganglia causes retardation of healing of perforating wounds of the ears of rabbits. In the present paper the healing of perforating wounds of the ear was studied in the same animal during stimulation of the central nervous system, namely of the cerebral cortex.

According to V. I. Baiandurov et al., [1], decortication of young animals has no essential effect on their growth, whereas injury to the corpora striata leads to marked delay in growth. O. S. Val'shonok [2] carried out destruction of fields 4 and 6 of one hemisphere and observed no difference in the healing of ulcers on the forelimbs. According to her results, there is no asymmetry in the healing of ulcers either after stimulation of field 4 by the application of a gauze swab soaked in strychinine. On stimulation of field 6 of the same hemisphere by application of gauze soaked in strychnine, a significant asymmetry was observed in all cases, as shown by the fact that an ulcer of the opposite limb reached its maximum development more rapidly and the period for it to heal was longer than in the case of its fellow. This author further points out the effect of stimulation could only be observed while layers 5 and 6 of the cortex remained undamaged. F. G. Popov [5] studied the healing of wounds of the skin in rabbits with injury to the cortex of one hemisphere and found considerable changes on the side opposite to the damaged hemisphere. These changes took the form of edema of the newly formed tissues, earlier maturation of the connective tissue and a tendency towards keratinization of the epithelium. V. A. Zhukhin and his co-workers [4] studied the course of inflammation and the healing of wounds of the skin in dogs after bilateral extirpation of the cerebral cortex with damage to the corpora striata. In this case the process of regeneration of the connective tissue and especially of the integumentary epithelium was retarded by comparison with controls and took place sluggishly. The numerous experiments carried out by V. G. Eliseev and his co-workers [3] showed that after partial extirpation of the cortex the processes of regeneration proceed quite well and injured internal organs recover, whereas if gauze is applied to the surface of the hemispheres healing of wounds takes place but not regeneration of typical organ tissue as such, although the macrophagic and fibroblastic reactions are intensified and the formation of a connective tissue capsule around a foreign body is accelerated.

EXPERIMENTAL METHOD

In the present investigation, carried out on 28 adult rabbits weighing about 2 kg each, we studied the healing of perforating wounds of the ears after the introduction of a foreign body into the subdural space of one cerebral hemisphere. The right ear (corresponding to the operation on the left hemisphere) was the "experimental" one and the left ear the control.

The foreign body used was thick x-ray film cleaned of its emulsion, from which a small plate measuring 1 × 2 cm was cut out; the plate was rounded, folded in two longitudinally and inserted under the dura mater. On the day after the operation standard puncture wounds 6 mm in diameter were inflicted on symmetrically placed areas of both ears. Areas of tissue from the margins of the wound were taken for histological examination on the 1st, 2nd, 4th, 8th, 10th and 16th day after infliction of the wounds. The material was fixed in 20% formalin, cut into sections with a refrigeration microtome, and the preparations stained with hematoxylin – eosin. In 12 rabbits macroscopic observations were carried out and the time of healing of the wounds recorded. Three of these rabbits died before the perforating wounds had healed and the degree of healing was judged by the size of the orifice which remained.

EXPERIMENTAL RESULTS

Histological study of the tissue of the wound margin 24 hours after its infliction showed that at this time a scab is formed along the margin of which there is a well marked leucocytic barrier. The connective tissue of the dermis, especially along the cartilage, is infiltrated by leucocytes throughout the whole extent of a 10 mm long section. The epithelium along the edge of the wound was separated from dead epithelium and formed a thickening. No difference was observed between the wounds of the right and left ears.

Two days after wounding the leucocytic barrier has become more massive, but infiltration of connective tissue in areas remote from the edge of the wound has diminished. The epithelial layer is thickened for a distance of 4 mm from the edge of the wound, and in places it consists of 6-7 layers of cells of the stratum germinativum and 2 rows of cells of the stratum granulosum, reaching 80 μ in thickness (normally the thickness of the epidermal layer here is usually no more than 20 μ and it is formed from 2-3 rows altogether of cells of the stratum germinativum and one incomplete row from the stratum granulosum). Epithelial wedges are formed and these infiltrate under the scab. We did not observe any difference between the conditions of the wounds on the right and left ears.

Healing of Perforating Wounds in Rabbits During Stimulation of the Cerebral Cortex

Serial No.	Rabbit No.	Time for healing of wounds (in days)		Ratio of healing time of right ear to healing time of left (in %)	Note
		right ear (experimental)	left ear (control)		
1	17	76	89	85	
2	18	26	29	89	
3	19	20	25	80	
4	20	0.5×0.5	1×3	—	Died after 30 days
5	21	22	24	91	
6	22	32	49	65	
7	23	2×2	4×4	—	Died after 30 days
8	24	29	35	83	
9	25	44	2×3	—	Observation ended after 86 days
10	26	44	44	100	
11	27	55	69	80	
12	28	5×6	7×7	—	Died after 30 days

Four days after wounding there is already considerable epithelial regeneration as far as the cartilage and, in some places, actually penetrating it. The leucocytic infiltration of the connective tissue is no longer evident; the formation of new, young connective tissue is seen, especially beneath the regenerating epithelium. Some delay is now observed in the growth of the regenerating epithelium in the left ear by comparison with the right.

Eight-ten days after wounding, in all cases the infiltration of epithelium under the scab is now finished

and the regenerating epithelium of the internal and external surfaces of the helix of the ear merge together. There is regeneration of the dermis over a variable length, projecting beyond the cut edge of the cartilage. There is a difference between this regeneration in the right and left ears: in the right ear the regeneration is seen to extend over a longer distance and is thicker.

Sixteen days after wounding areas of regeneration are seen to project far beyond the cut edge of the cartilage, and in all cases the length of the regenerating area is greater in the right ear.

Macroscopic observations showed that in 11 out of 12 cases the wounds on the right (experimental) ear healed more quickly, although the differences were variable, as can be seen from the Table. Of 8 rabbits in which the perforating defects closed in both ears, in 7 cases closure of the orifice took place more rapidly on the right, on the side opposite to the operation, but in one case the wound defects closed simultaneously after 44 days. In three cases the wounds did not succeed in closing on either ear, but the residual orifice was smaller on the right ear. Finally in one rabbit the defect on the right ear closed after 44 days, and on the left ear at this time an orifice remained open, measuring 2-3 mm, which persisted until the 86th day when the observations were discontinued.

Thus the experimental results showed that the insertion of a foreign body in the subdural space does not lead to asymmetry in the course of the wound in the first few days. Starting on the 4th day, and becoming more marked subsequently, a speeding up appears in the regenerative process on the side opposite to the cerebral hemisphere on which the operation was performed. The microscopic findings are in agreement with the results of the healing times of the wounds.

The results of this investigation are similar to those of our previous work [8] on the study of healing of wounds in rabbits after partial decortication.

The resemblance between the results obtained suggests that both extirpation of a small area of cortex and the insertion of a foreign body into the brain tissue in our experiments led to the same physiological effect, i.e. to stimulation of the residual, larger area of cortex, and causing activation of the processes of regeneration. The possibility of such activation is also shown by the results of our investigations (Prizhivoit and Aubakirov [9]), in which it was shown that stimulation of the cerebral cortex by injection of caffeine accelerates the course of regeneration in the skin.

Our findings are in agreement with the observations of Popov (1954), who established the presence of asymmetry of the processes of regeneration after unilateral trauma to the forebrain in rabbits. At the same time they differ from the findings of O. S. Val'shonok [2]. This may possibly be due to the fact that O. S. Val'shonok's work was done on dogs, and more important, she used stronger forms of stimulation which could affect not only the cortex of one hemisphere but also the opposite cortex and the subcortical ganglia.

In our experiments the rate of closure of the perforating defect of the ear was determined by the processes taking place in the connective tissue. The marked acceleration of these processes during stimulation of the cortex is in agreement with the findings of V. G. Eliseev [3] of acceleration of the fibroblastic reaction and the more rapid formation of a connective tissue capsule around a foreign body under similar conditions.

The same type of effect on the process of regeneration in rabbits after removal of a part of the cortex and the application of a gauze swab was observed by A. A. Savinovskaia [10], who studied the mitotic activity in the mesothelium after the injection of air into the peritoneal cavity, and found that chronic irritation had a more pronounced effect than partial decortication. These results are in agreement with our own observations.

Our experiments have thus shown that by stimulation of the cerebral cortex of adult rabbits it is possible to influence the course of the process of regeneration (the healing of wounds of the skin) and that disturbance of cortical activity does not cause any retardation in the process of regeneration, as we have seen after disturbance of the peripheral innervation [16, 17], but may even lead to acceleration of regeneration.

SUMMARY

The author studied the healing of penetrating wounds in the rabbit's ears. He inserted a foreign body, a piece of x-ray film, under the dura mater on the surface of the left cerebral hemisphere of these experimental animals. A quicker healing of the wounds was noted on the right side, as compared with the left (i.e. on the side innervated by the mechanically stimulated cerebral hemisphere).

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MORPHOLOGY AND PATHOMORPHOLOGY

A HISTOCHEMICAL STUDY OF THE CHOLINESTERASE ACTIVITY OF THE NERVE CELLS AND CONDUCTING SYSTEM OF THE HEART

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During recent years there has been considerable development of histochemical methods of investigation, which enable the study of the histological and cytological structures to be approached from the point of view of the localization therein of various chemical components. For this reason the results of investigations using histochemical reactions are undoubtedly important for a proper understanding of the metabolic processes taking place in these structures.

The aim of the present investigation was to study the localization and degree of activity of the specific cholinesterase in the Purkinje fibers of the bundle of His in the conducting system of the heart, and also in the structures of the nervous system of the interventricular septum. We were particularly interested in the conducting system since this is known to differ only slightly in its histological structure in the majority of animal species from the rest of the heart muscle, although it has a special function.

EXPERIMENTAL METHOD

We studied the interventricular septum of the heart in the sheep, ox and pig, i.e. in animals in which the conducting system of the heart is particularly well expressed and suitable for study. Material was taken from animals only recently killed, placed in cold physiological saline, and dealt with not later than 30 minutes afterwards to avoid spontaneous diffusion of the enzyme. In order to reveal the activity of specific cholinesterase we used Koelle's method [2] which is based on the enzymic hydrolysis of the thioanalog of acetylcholine – acetylthiocholine – with the production of a precipitate of free thiocholine in the form of its copper mercaptide, and subsequent conversion of this into CuS. Copper sulfide, precipitated in cholinergic structures in quantities depending on the degree of activity of the enzyme, gives them a brownish color which varies in intensity from almost black to yellow. To minimise the possibility of diffusion of cholinesterase, the reaction was carried out with a 24% solution of sodium sulfate. Sections were cut to a thickness of 60 μ with the knife of a freezing microtome and placed for 30 minutes in a 10^{-7}M solution of di-isopropylfluorophosphate to inhibit the nonspecific cholinesterase; they were then transferred to glass slides and incubated for 1 hour 45 minutes in a medium at pH = 6.4.

In relation to the innervation of the bundle of His our observations confirm the findings of other workers [3].

EXPERIMENTAL RESULTS

Cholinergic nerve fibers or bundles of nerve fibers proceed in the layers of loose connective tissue between the Purkinje fibers, lying close to them and enveloping them on all sides. Anastomosing with each other, branching and altering the direction of their course, they form a single network of coarse loops; this can be

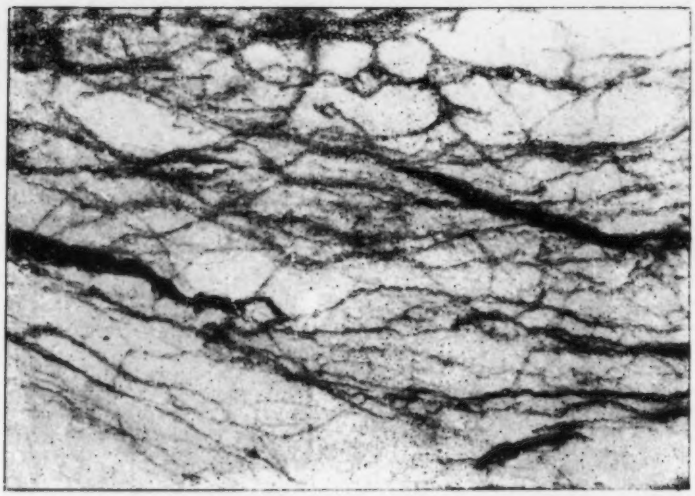

Fig. 1. The bundle of His from the heart of a sheep. Purkinje fibers, colorless and negative in relation to specific cholinesterase, are intertwined with highly active nerve fibers. Magnification 15 × 6 × 1× 4.

observed with particular clarity in the bundle of His in the sheep and ox. Larger nerve trunks are also encountered, in whose path separate nerve cells may be seen.

All the nerve fibers and cells are highly active in respect of specific cholinesterase, as shown by the intensity of their staining. The separate trunks and fibers are almost black in color, which distinguishes them from the general mass of fibers forming the plexus; the majority of these fibers are stained a brown color, and the separate fine fibers brownish yellow. It is interesting that the Purkinje fibers themselves have negligible cholinesterase activity and appear in the films almost colorless (Fig. 1).

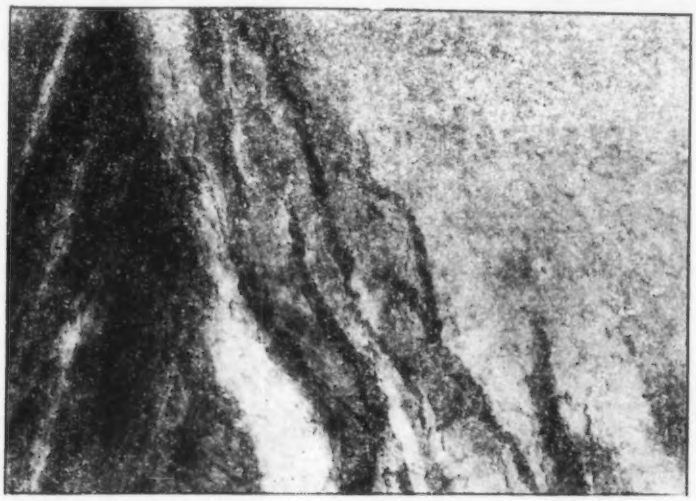

Fig. 2. Heart of a pig. Preparation counterstained with hematoxylin. The topographical relationships between the Purkinje fibers and bundles of nerve fibers passing between them are seen. Magnification 8 × 6 × 1 × 4.

According to our observations the muscle fibers of the bundle of His have an even lower cholinesterase activity then the surrounding cardiac muscle tissue, i.e. they are practically without enzyme. The topographical relationships between the individual Purkinje fibers and their accompanying nerve fibers are clearly seen in the preparations counterstained with hematoxylin. In Fig. 2, in the heart of a pig, clearly outlined thick Purkinje fibers can be seen, anastomosing with each other, and with bundles of nerve fibers passing between them.

The brown nerve fibers are clearly distinguishable against the pale violet background of the counterstained Purkinje fibers, showing no signs of the presence of cholinesterase. In this respect we are quite unable to agree with the findings of Mohr [4], who studied the cholinesterase activity of the conducting system of the heart in guinea pigs, rabbits and the ox by means of the method of Koelle and Friedenwald as modified by Gerebtzoff [1], and who came to the conclusion that the conducting system is positive in respect of specific cholinesterase and that the enzyme envelops each Purkinje fiber in the form of a muff. In this case it is evident that diffusion of the enzyme took place, and on transverse section the divided nerve fibers, closely intertwined with the fibers of the conducting system, created the impression of a continuous ring.

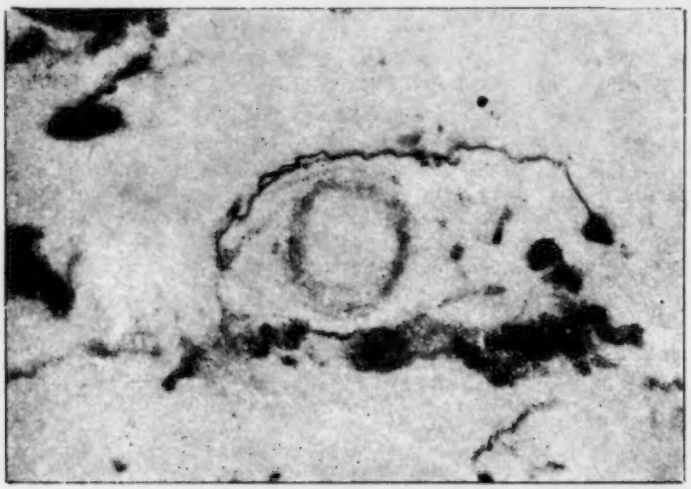

Fig. 3. Heart of a sheep. Side by side with a vessel lie single nerve cells with high cholinesterase activity. Processes from these cells, surrounding the vessel lose themsleves in its adventitia. Magnification 15 × 6 × 1 × 4.

The muscle tissue of the upper third of the interventricular septum is rich in its innervation. Fine nerve fibers run parallel with the muscle fibers, divide dichotomously, intertwine and form a continuous plexus. Individual nerve endings may also be met. Unfortunately the pale brown nerve fibers do not stand out well against the yellowish background of the muscle tissue, and this creates certain difficulties when taking microphotographs. In the lower third of the septum we found considerably fewer nerve fibers. In the upper part of the interventricular septum single microganglia and individual nerve cells are encountered in the layers of loose connective tissue. The cells of these ganglia stain with varying intensity – from almost black to light brown, which suggests different degrees of cholinesterase activity. Nerve fibers leave these ganglia, but it was not possible to trace them for any considerable distance. The nuclei of the nerve cells of these ganglia are not active in respect of specific cholinesterase; they are colorless in the sections.

In the sheep's heart, as a regular feature, microganglia were observed to be localized around small vessels and vessels of larger caliber. Single nerve cells could occasionally be seen lying near a vessel. When the course of the processes of these cells could be traced, it could be observed that they run towards the vessel, go around it, become thinner and lose themselves in its adventitia (Fig. 3).

The nerve cells of the ganglia and the nerve fibers show a different intensity of staining, i.e. unequal cholinesterase activity, which may possibly be associated with their differing functional state.

SUMMARY

The localization and the degree of activity of the specific cholinesterase was studied in the Purkinje fibers of the bundle of His of the cardiac conductive system, as well as in the nerve structures of the interventricular septum in sheep, hogs and oxen.

G. Koelle's method was used for the detection of the specific cholinesterase. It was established that Purkinje's fibers are richly innervated. The cholinergic nerve fibers form a continuous network and possess a high cholinesterasic activity. The Purkinje's fibers themselves remain negative with relation to the specific cholinesterase. Interventricular cardiac septum, especially in its upper third, contains numerous delicate nerve fibers with a low cholinesterase activity. The intramural nerve ganglia consist of the nerve cells with a different degree of cholinesterase activity, which is usually high. The groups of nerve cells in the myocardium are usually localized around the small and large blood vessels.

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HISTOPATHOLOGICAL CHANGES IN ANIMALS IN THE PRESENCE OF ANAEROBIC INFECTION AND WHEN SUBJECTED TO WEAK STIMULATION

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In previous investigations [4, 5] were demonstrated certain characteristics of the neurodystrophic process in guinea pigs infected with B. perfringens when the nerve supplying the infected muscle is affected.

In the current project carried out on white rats, the neuroreceptor apparatus of the infected muscle was subjected to additonal action.

EXPERIMENTAL METHOD

A minimal lethal dose of vibrion septique mixed with a 2.5% solution of calcium chloride was administered into the gastrocnemius muscle of experimental rats. Twenty-four – 3 hours prior to infection or 1-3 hours following it, 0.1 ml of 0.25% solution of a stable preparation of novocain in apricot oil was injected into the same muscle or 0.1 ml of sterile apricot oil was injected 24 hours prior to infection. Control animals not subjected to supplementary intervention • were infected at the same time as the experimental animals. Three series of experiments were carried out on 118 rats.

EXPERIMENTAL RESULTS

The experimental results are presented in Tables 1 and 2.

In order to establish the effect of apricot oil on the pathogenicity of microbes, a third series of experiments was carried out the results of which are presented in Table 3.

Thus, injection of novocain or apricot oil in the same muscle that was infected, especially when administered 24 hours prior to the infection, prevents death of some of the animals and at times of the majority. Direct contact of the microbes with the apricot oil does not influence their pathogenicity significantly.

Observations indicated that the paw into which the microbes were injected showed involvement of a lesser degree in animals given apricot oil or novocain 24 hour prior to infection than in others. Histopathological examination of both gastrocnemius muscles and both sciatic nerves was performed in 14 rats of the second series of experiments sacrificed on the 1st, 7th and 45th day after infection. Sections were stained with scarlet red for fat and counterstained with hematoxylin.

Microscopic examination of the preparations showed the following. In one day after infection of rats of the fourth group (control) the right gastrocnemius muscle was found to be overrun by microbes.

•Infection of the animals was carried out by co-worker Z. I. Sobieva of the Laboratory of Infectious Pathology.

TABLE 1

The First Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished
		total	perished	survived	
First	Injection of novocain into the right gastrocnemius muscle 24 hours prior to infection	10	1	9	27 hours — 1 rat
Second	The same 3 hours prior to infection	10	7	3	20 " 1 " 22 " 4 rats 24 " 1 " 43 " 1 "
Third	The same 3 hours after infection	10	6	4	19 hours 2 rats 22 " 2 " 27 " 1 rat 43 " 1 "
Fourth	Infection without supplementary intervention (controls)	10	8	2	19 hours 2 rats 22 " 5 " 43 " 1 rat

TABLE 2

The Second Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished
		total	perished	survived	
First	Injection of novocain into the right gastrocnemius muscle 24 hours prior to infection	10	6	4	12 hours — 1 rat 24 " 3 rats 4 days 2 "
Second	The same 1 hour after infection	10	5	5	12 hours 2 rats 22 " 1 rat 24 hours 2 rats
Third	Injection of apricot oil into the right gastrocnemius muscle 24 hours prior to infection	10	3	7	12 hours 2 " 4 days 1 rat
Fourth	Infection without supplementary intervention (controls)	10	7	3	12 hours 4 rats 24 " 3 "

TABLE 3

The Third Series of Experiments

Group	Infection method	Number of animals			Life term of the animals that perished following infection
		total	perished	survived	
First	Infection of apricot oil into the left gastrocnemius muscle 24 hours prior to infection of the same muscle	10	5	5	22 hours – 1 rat 24 " 2 rats 27 " 1 rat 4 days 2 rats
Second	Injection of apricot oil into the left gastrocnemius muscle 24 hours prior to infection of the muscle of the left thigh	10	5	5	22 " 5 rats
Third	Injection of a mixture of microbial suspension and apricot oil into the left gastrocnemius muscle	9	7	2	22 " 2 rats 24 " 2 " 27 " 2 " 42 " 1 rat
Fourth	Infection without supplementary intervention	9	9	0	22 " 8 rats 72 " 1 rat

Edema of the intermuscular connective tissue, fragmentation, swelling and fatty degeneration in many muscle fibers and hemorrhage are very much in evidence (Fig. 1). The numerous foci of round cell infiltrates, consisting of lymphocytes and to a lesser extent of polymorphonuclear leucocytes, contain numerous degenerated cells. No mitoses were seen in the infiltrates or uninvolved tissue. Nuclear proliferation, fatty degeneration of individual muscle fibers, and infrequently – small foci of round cell infiltrates, were observed in the left gastrocnemius muscle. The right sciatic nerve showed that the epineurium, was thickened, hyperemic and contained large foci of round cell infiltrates. There were no changes in the left sciatic nerve.

In the right gastrocnemius muscle of the experimental animals were seen the same destructive changes as seen in the control rat, but they were observed in a few muscle fibers. In the uninvolved muscle tissue of these animals, proliferation of muscle and connective tissue nuclei was less marked than in control rats and mitoses were encountered. At the site of injection of novocain or oil there was a cavity containing a considerable quantity of oil. In this cavity as well as in the surrounding area there were collections of round cells which were bound with cellular matrix to round cell infiltrates in other portions of the sections (Fig. 2). In distinction from findings at examination of analogous preparations from control animals, these infiltrates of lymphocytes and polymorphonuclear leucocytes also contained many polyblasts and macrophages among which not infrequently one could find cells with mitotic figures. Nuclear proliferation and at times mitoses could be seen in the left gastrocnemius muscle. In this muscle, areas of fatty degeneration along the course of muscle fibers as well as vascular hyperemia and proliferation of perivascular connective tissue are seen on occasion. Only in the rat from the second group could foci of infiltrates within the substance of the nerve be seen in the right sciatic nerve; hyperemia and fresh hemorrhages in the epineurium were found in the left sciatic nerve.

Seven days following infection (Fig. 2) of the right gastrocnemius muscle of the control rat, hyperemia with thickening of the vessel walls and proliferation of connective tissue replacing involved muscle tissue could

be found. Fatty degeneration of muscle fibers was rather mild. The right sciatic nerve was hyperemic and adherent to the adjacent tissues. In the left gastrocnemius muscle were found hyperemia and proliferation of perivascular connective tissue; in the left sciatic nerve – hyperemia and proliferation of connective tissue elements in its coats.

In experimental rats of the first and third groups, also 7 days following infection, there was replacement of part of the muscle tissue of the right gastrocnemius muscle with granulation tissue and numerous foci of round cell infiltrates. Here were present fatty degeneration of muscle coats and myelin coats of the nerve fibers as well as macrophages loaded with fat and pigment. At the site of injection of novocain and oil there is a slit-like space filled with fat and surrounded by a layer of lymphoid, histiocytic and other connective tissue elements. In the preserved muscle tissue one can observe in some fields of vision proliferation of muscle nuclei and connective tissue elements among which occasional cells with mitotic figures are present. Proliferation of adipose cells is marked, especially along the course of vessels and nerves. In the left gastrocnemius muscle one can see now and then fatty degeneration of muscle fibers and hyperemia. Proliferation of adipose cells, of muscle nuclei in chain-like arrangements and of connective tissue cells which in places obscure portions of muscle fibers are conspicuous. In portions of the right sciatic nerve the epineurium is thickened and adherent to the surrounding tissues; now and then focal collections of endoneural nuclei are encountered. In the left sciatic nerve only an insignificant thickening of the epineurium is observed.

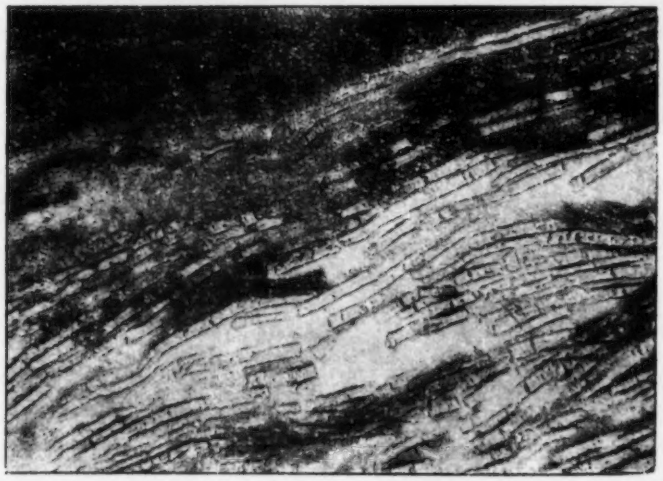

Fig. 1. Condition of muscle tissue (right gastrocnemius muscle) of a control rat sacrificed 24 hours after infection. Destructive changes in muscle tissue are evident. Fat stain. Microphotogram 67.2 x.

In the rat of the second group the histopathological picture of the muscles and nerves resembels the one just described. The difference is in the greater intensity of the destructive process in the right gastrocnemius muscle where structureless fragments of muscle fibers are surrounded by giant cells, histiocytes, macrophages and fibroblasts. The entire area of involvement is delimited from the remaining muscle tissue by a roller of round cells.

On the 45th day after infection, proliferation of connective tissue cells (including massive proliferation of connective tissue) of muscle nuclei and adipose cells in both muscle and coats of both nerves is more pronounced in experimental than in control rats.

It is obvious from the cited microscopic findings that during the first day after infection the less striking destructive changes in the muscles and nerves characteristic of the pathological process being studied were observed in rats of the first and third groups; they increased in the second group and reached the maximum degree in animals of the fourth group (controls). This agrees with findings observed in the involved extremity which were made while the animals in this series of experiments were still alive.

The preparations indicate that the pathological process in the region of infection differed in the experimental and control animals in the degree of involvement of muscle tissue and in the character of reaction on the part of tissue. The latter, apparently, determined the outcome of the infection and the subsequent course of the processes of restoration. As compared to controls, the experimental animals exhibited a lesser degree of destructive changes in the muscles and a greater degree of activity on the part of mesenchymal elements including adipose cells.

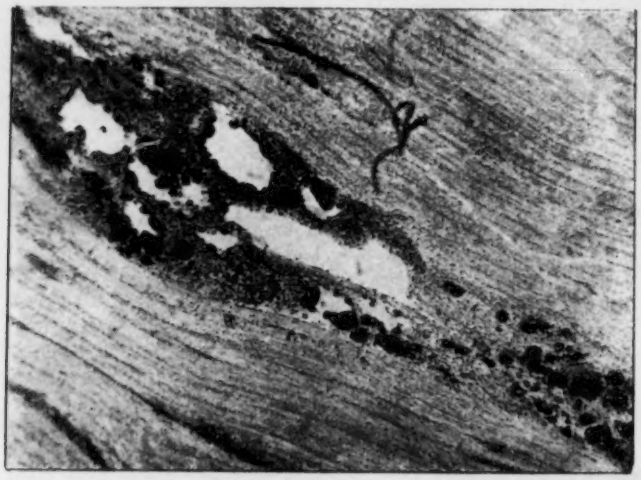

Fig. 2. Condition of muscle tissue (right gastrocnemius muscle) of a rat sacrificed one day following infection. Relative preservation of muscle tissue at the site of injection of apricot oil is evident. Fat stain. Microphotogram 67.2 ×.

In the literature of recent years dealing with the problem of anaerobic infection, more and more attention is being paid to the reactivity of the organism which is associated with the functional condition of the nervous system. Academician A. D. Speranskii's position on the leading role of the nervous system in pathological and protective and adaptive processes was confirmed by the investigation of the mechanisms of development of gas gangrene [1, 2, 3, 6]. The same authors pointed out the favorable action of novocain as an anesthetic substance on the outcome of experimental infection with microbes and toxins of gas gangrene.

Reactions in the form of proliferation of muscle nuclei, appearance of adipose cells, and connective tissue and blood elements were observed by us not only in the muscle into which novocain, oil and microbes were injected, but in the contralateral – so-called healthy muscle and in the coats of its nerves. Consequently, it is possible to speak of general stimulation of proliferative processes in mesenchymal tissues by a nonspecific irritation. An organism prepared in such a manner may be found to be resistant to a lethal dose of vibrion septique. Under such conditions the pathological process in the focal lesion loses in intensity, which, as our earlier observations indicated, favors a decrease in changes in the nervous system.

SUMMARY

A culture of vibrion septique was injected into the gastrocnemius of white rats 24 or 3 hours previous to the infection or 1 to 3 hours following it a stable preparation of novocain in apricot oil or apricot oil alone was injected into the same muscle. Control rats were injected simultaneously, but without any additional interventions. The outcome of the infection was more favorable in the experimental rats. The injection of novocain or apricot oil, especially carried out 24 hours before the infection prevented the death of a number, at times of the majority of the animals. The extremity into which the microbe was injected was found to be affected less in animals in which novocain solution or apricot oil was injected 24 hours previous to the infection (in comparison with the rest of the animals). The microscopic examination of both gastrocnemius muscles and

both sciatic nerves demonstrated that novocain or apricot oil injection brought about a decrease in the destructive changes in the affected muscle and an intensification of the reactive processes in the mesenchymal elements. The latter, evidently, promoted an increase of the resistance of the animals to the lethal dose of vibrion septique.

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THE STATE OF THE RECEPTOR APPARATUS IN THE WALLS OF CERTAIN MAJOR BLOOD VESSELS AFTER IRRADIATION BY X-RAYS

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In papers by Soviet scientists numerous findings have been reported, developing the teaching of I. P. Pavlov on the role of the nervous receptors in pathological conditions. Of great importance here are morphological investigations of the state of the receptor apparatus in various pathological processes. In this direction work has been successfully carried out for many years in the laboratory directed by B. A. Dolgo-Saburov. V. V. Kupriianov [5], for instance, discovered well marked paranecrotic changes in the afferent apparatus during experimental oxygen lack. On the basis of his investigations this author came to the conclusion that the reaction in the afferent endings arises sooner than in the nerve cells themselves. Similar findings were obtained by B. A. Dolgo-Saburov [3] after abolition of efferent activity and by V. V. Astakhova [1] during experimental fever.

In 1954, a start was made on a morphological investigation of the receptors of certain major vessels after the action of x-rays. Many authors [2, 6, 7, 8] have demonstrated marked reflex disorders appearing after x-ray irradiation. These disorders form one of the main links in the pathogenetic chain of development of acute radiation sickness. In view of this it appeared important to ascertain the state of the structure of the interoceptors after the action of ionizing radiation.

EXPERIMENTAL METHOD

At the beginning of our investigation we studied the nervous apparatus in the wall of certain major blood vessels in animals exposed to the action of large doses of x-rays. The material used in the investigation comprised the carotid sinus zone, the venae cavae and the pulmonary and portal veins. These vessels were fixed in 12% neutral formalin solution for 12-14 days, stained by the Bielschowsky – Gros method and later counterstained with hematoxylin – eosin.

Material from 10 cats was studied. Before irradiation careful observations were carried out on the cats for a period of 15 days, including measurement of the temperature and body weight and full blood count; only healthy male animals were selected for the experiment. After this procedure the animals were irradiated with a dose of 600 r.

On the 3rd day after irradiation the general condition of the cats was good, but they showed considerable changes in the composition of the peripheral blood in the form of leucopenia with a relative and absolute lymphocytopenia, characteristic of the latent period of acute radiation sickness. The animals were killed at this period and material taken for histological examination.

EXPERIMENTAL RESULTS

As a result of the study of the preparations it was established that on the 3rd day after irradiation various changes could be observed in the nervous apparatus in the walls of certain major blood vessels, ranging from increased argentophilia to total destruction of the terminal apparatus.

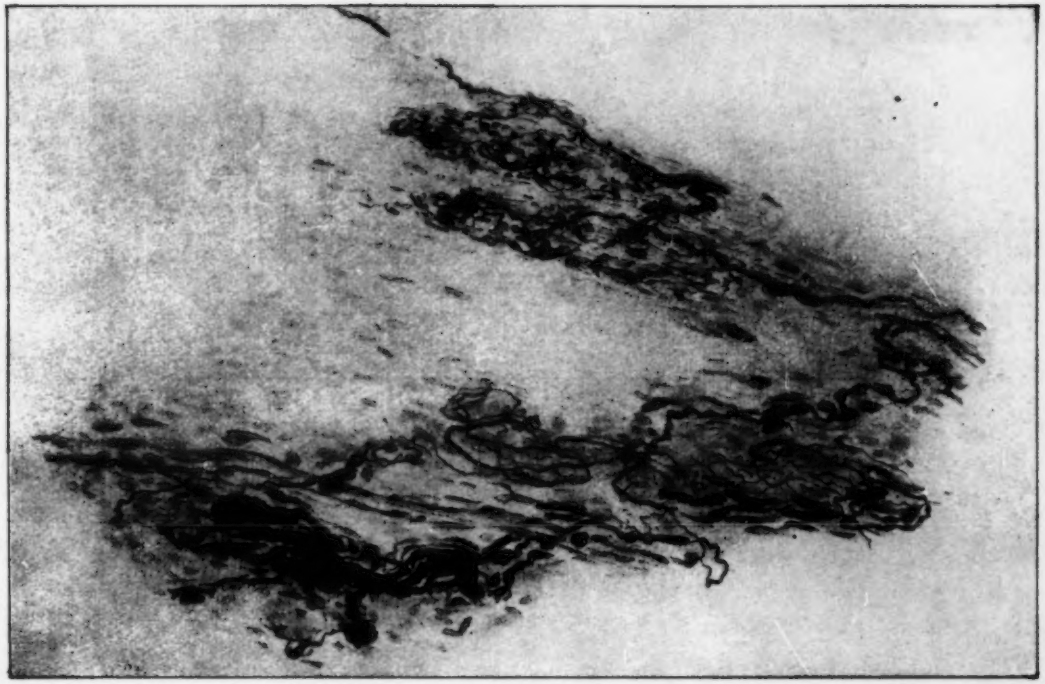

Fig. 1. Severe changes in the lower receptor with hypertrophy of all its structures. In the upper receptor there are signs of destruction with rupture of loops and of end-bulbs and varicose dilatations of the preterminal segment of the nerve fiber. Bielschowsky – Gros method. Magnification: ocular 10 x, objective 40 x.

Fig. 2

Fig. 3

Fig. 2. Modified receptor in the wall of the anterior vena cava. Disintegration of terminal structures, fragmentation of the conducting fiber and formation of poorly staining spheres of large size are seen. Bielschowky – Gros staining. Magnification: ocular 15 ×, objective 40 ×.

Fig. 3. Modified receptor in the wall of the anterior vena cava. Varicose dilatations and swellings of the nerve fiber seen in its preterminal part, widespread hemorrhage in the zone of the receptor. Bielschowsky – Gros staining. Magnification: ocular 10 ×, objective 40 ×.

In the first place ease of impregnation of the nerve cells and an abundance of nerve cells in the preparations were revealed. Thanks to the high argentophilia an extremely intensive staining in a black color of the receptor apparatus was usually noted, with well differentiated fine structural details.

One of the most common variants of the reactive changes in the receptor endings after irradiation is hypertrophy of the structures of the terminal apparatus. In these cases the terminal endings appear as intensively and homogeneously stained varicose dilatations. Figure 1 illustrates a group of receptor endings in the wall of the anterior vena cava. A severe change is clearly seen in the receptor, with hypertrophy of all its structures; in another ending there are significant changes with signs of destruction, rupture of loops and end-bulbs, and varicose dilatations of the preterminal part.

In addition to increased argentophilia and excessive growth of the terminal endings, the destruction of the terminal structures which is quite often seen must also be regarded as an extremely characteristic change.

Signs of destruction of the receptor endings were observed in the venae cavae, the pulmonary veins and the carotid sinus zone; changes of this sort were not observed in the portal vein.

Figure 2 illustrates a sharply altered receptor with destruction of its terminal structures and fragmentation of the conducting nerve fiber with formation of badly staining fragments of large size.

We must emphasize particularly the description of the state of the afferent fibers in their preterminal part. In these segments of the peripheral nerve fibers are seen the most pronounced changes in the form of a coarsening of the nerve fibers, the formation of extensive accumulations of neuroplasm along their course, resembling globular or spherical swellings, and fragmentation with loss of connecting traces between the separate fragments.

In Fig. 3 is shown a severely modified receptor in the wall of the anterior vena cava with varicose dilatations and hypertrophy of the nerve fiber in its preterminal part. Similar reactive changes are also observed in the medullated nerve fibers of the carotid sinus zone. Here, in many of the receptor endings the preterminal part appears as coarse, intensively stained fibers, black in color, with collections of neuroplasm and extensive varicose dilatations. Some fibers terminate in pin-head swellings with no proper endings, others are broken up into separate fragments, sometimes taking on the appearance of enormous spheres.

During the study of the preparations attention was drawn to the presence in the majority of severe dilatation and distension with blood of the capillaries, with the formation of extensive extravasation of blood into the tissues (see Fig. 3).

It must be emphasized that the morphological changes in the nerve endings described above were met only in the venae cavae, the pulmonary veins and the carotid sinus zone; in the wall of the portal vein the receptor endings were by no means so appreciably modified.

From the results which we obtained it is clearly seen that on the 3rd day after irradiation paranecrotic changes appear in the preterminal and terminal portions of the afferent nerves in the walls of the venae cavae, the pulmonary veins and the carotid sinus zone. These changes are essentially similar to those which have previously been seen by many other workers in various pathological conditions [1, 2, 3, 4, 5], and they are evidently nonspecific in character.

SUMMARY

Experiments were performed on cats. The author studied the condition of receptor apparatus in a number of large blood vessels at the early periods following x-ray irradiation. Pronounced reactive changes of receptors were revealed in the wall of the venae cavae, the pulmonary veins and the synocarotid zones on the third day following the irradiation with the dose of 600 r. These changes were manifested in various morphological pictures from increased argentophilia to complete disintegration of the terminal apparatuses. These changes in the receptors are analogous to those observed formerly by a number of authors in other pathological conditions and are, evidently, nonspecific in character.

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*In Russian.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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